CLINICAL AND EXPERIMENTAL

A STUDY OF THE SPECIFICITY OF THE KOLMER COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By John A. Kolmer, M.D., D.Sc., and Edward Steinfield, M.D.

IN all probability the complement-fixation test for syphilis serves its most useful purpose in the diagnosis of the disease in its clinically latent and tertiary stages, as a guide in treatment and as one criterion of cure. In the primary stage it may, of course, aid in the diagnosis of clinically atypical infections when employed along with dark-field examinations for *Spirochete pallida* and likewise confirm the clinical diagnosis in the secondary stage and greatly aid in the diagnosis of atypical cases at this stage of the disease, but we believe that most physicians will agree with us that the complement-fixation test is, or, at least should be, of most service as an aid in the diagnosis of the clinically obscure, doubtful and unsuspected cases of acquired and congenital syphilis in which even a most careful and exhaustive history and physical examination may yield inconclusive and doubtful results.

The very nature of the pathologic and immunologic changes in cases of chronic and latent cases of syphilis demands that the complement-fixation test shall be as sensitive as consistent with specificity to render its best service as an aid in diagnosis, as a guide in treatment and as a criterion of cure. In the first place there are reasons for believing that the antibody-like substance occurring in the blood and spinal fluid responsible for complement fixation in syphilis is a product of the lymphocytes and other cells in more or less direct contact with *Spirochete pallida* in the tissues rather than a product of stimulation of the bone marrow and other distant antibody producing

organs by a diffusible antigen as occurs in most other infections. There are no reasons at all for regarding this antibody-like substance as a true antibody in the sense of being destructive for *Spirochete pallida*; rather it is a by-product of cellular stimulation or degeneration rendering the complement-fixation reaction in syphilis a measure of the degree of infection rather than of the degree of immunity. If, however, the strength or degree of complement fixation in syphilis with lipoidal extracts is an index of the degree of spiro-chetic activity, there are numerous instances in which the reaction is quite marked in cases without coincident clinical evidences of activity, but it is to be kept in mind that the signs and symptoms of chronic syphilis are due largely to the location of the spirochetes and the degree of involvement of physiologically important tissues. For example, a relatively few spirochetes in the posterior columns of the spinal cord may be sufficient for the produc-tion of marked signs and symptoms of locomotor ataxia; the numbers of parasites may be indeed too few to produce sufficient of the antibody-like reagin to yield positive complement-fixation reactions and an equal number of spirochetes in a less important tissue like the periosteum of a long bone, may produce no discernible clinical signs and symptoms at all. On the other hand a larger number of spirochetes scattered about in the heart muscle and walls of the larger blood vessels or elsewhere may produce relatively large amounts of "reagin" yielding strongly positive Wassermann reactions with-out necessarily being accompanied by a commensurate degree of physical disability and sickness. We think it is important for physicians to keep this possibility in mind as affording a reasonable explanation for the occurrence of truly positive Wassermann reactions with the sera of individuals without discoverable clinical evidences of syphilis.

If, therefore, we are correct in our surmise that the "reagin" or "alexo fixagin"* in the blood and spinal fluid of cases of syphilis is produced by the lymphocytes and other cells in contact with *Spirochete pallida* and its prod-ucts in the tissues then we must be prepared to admit that the reaction may be negative in such cases of syphilis in which the spirochetes are so latent that they exist in the tissues with little or no cellular reaction as indicated by the histologic studies of Warthin and his colleagues. We must also admit at the same time that our complement-fixation test must be as sensitive as consistent with specificity in order to detect as many such cases as possible and especially since they may quite easily escape clinical diagnosis. Their detection may not necessarily indicate the institution of active antisyphilitic treatment: this is a separate question not directly concerned in the present discussion, but they nevertheless require our best diagnostic efforts and especially when the complement-fixation reaction is being employed as an aid for determining cure in order to avoid the very regrettable error of under-treating the disease since such spirochetes may suddenly and unexpectedly spring into renewed activity at subsequent periods in the individual's life. We believe therefore that even with the most sensitive methods there will always occur a certain unknown percentage of falsely negative Wassermann

*A term employed by one of us (J. A. K.) for designating the complement or alexin fixing type of antibody.

reactions in cases of extremely latent syphilis because of insufficient amounts of "reagin" in the blood. But it is the plain duty of serologists to employ methods as sensitive as consistent with specificity to reduce to a minimum the percentage of these falsely negative reactions. We are convinced that efforts of this kind require unremitting attention and a high degree of technical skill, but when one pauses to consider for a moment the potential importance of the majority of Wassermann tests to the individuals concerned, to members of their immediate family and to the state, it is readily realized that the complement-fixation test for syphilis is by all odds the most important routine procedure today in medical laboratory practice worthy of our best efforts and in the conduct of which mere economy of time and materials and the desire for the return of quick reports are certainly much less important than the accuracy of results. An error in urinalysis, a blood count, a bacteriologic examination, etc., does no particular harm and if it has made an impression upon the patient and his physician is readily corrected, but an error in the Wassermann reaction and especially the occurrence of a falsely positive reaction, may be even more serious than an erroneous diagnosis of cancer in a tissue leading to a dangerous operation.

In our efforts to render the complement-fixation test for syphilis as sensitive as consistent with specificity we may run into the danger of nonspecific falsely positive reactions, but these are avoidable by correct technical procedures. Likewise errors in technic may occur as long as the test is conducted by human hands although the incidence of these can be reduced almost to a negligible minimum by confining the conduct of the tests to responsible and skillful workers but the very important question not infrequently arises, do changes occur in the blood in nonsyphilitic diseases that may lead to falsely positive reactions with technically acceptable methods? In other words may an antibody-like substance occur in the blood in diseases other than syphilis capable of causing the flocculation of lipoids in colloidal suspension with the absorption or fixation of complement similar to the mechanism of the Wassermann reaction? This is a question of fundamental importance because it concerns the possibility of the occurrence of biological, and not technical, falsely positive reactions.

We think it is to be granted that in trypanosomiasis, leprosy and possibly in a few other febrile diseases that changes *may* occur in the colloidal chemistry of the blood which increase the tendency of the serum to yielding nonspecific reactions by a mechanism similar to that responsible for the occurrence of positive Wassermann reactions with the sera of healthy rabbits, dogs and mules. The mechanism of this kind of reaction is not known and it is hardly necessary at this time to attempt offering an explanation, but the phenomenon is not merely one of anticomplementary action of serum so readily detected in the serum control tube. It is rather of a nature similar to the proteotropic reaction of Noguchi which refers to falsely positive Wassermann reactions with unheated normal human serum due to the fixation of complement by some interaction between the serum and the proteins of alcoholic tissue extracts employed as antigens. In other words these falsely

positive reactions are avoidable by the use of superior antigens and such technical procedures as enhance the degree of specific complement fixation by syphilis "reagin" while depressing the degree of nonspecific fixation by serum alone, antigen alone or a combination of antigen and various constituents present in all sera apart from the syphilis reagin.

Experience with the new complement-fixation test for syphilis devised by one of us (J. A. K.) during the past three years and covering many thousands of reactions reported by investigators in different parts of the country have established that the new method when properly conducted possesses three outstanding properties, namely, (a) a very high degree of specific sensitiveness; (b) an almost total absence of tendency for yielding apparently nonspecific reactions and (c) a very low percentage of anticomplementary reactions. We believe these results are largely due to four factors, namely, (a) to the employment of a new antigen which permits the use of a dose of 10 antigenic units which amount is yet 30 to 50 times less than the anticomplementary unit; (b) to the employment of a period of primary incubation of 18 to 20 hours at 6 to 8° C. followed by 10 minutes at 38° C. which greatly favors the fixation of complement by syphilis "reagin" and this extract while minimizing the degree of fixation by serum alone and extract alone; (c) to the use of an antisheep hemolytic system which permits the use of small amounts of guinea pig serum complement and rabbit antisheep hemolytic serum and thereby reducing to a minimum the presence of various serum constituents capable of interfering with specific complement fixation and (d) to the use of varying amounts of patient's serum which in a small but appreciable percentage of cases increases the specific sensitiveness of the test. As previously stated it cannot be hoped nor is it expected, that the new method is proof against technical errors but we believe that when it is properly conducted with an acceptable antigen that the positive reactions in this country are due alone to syphilis since frambesia or yaws is practically unknown.

<center>TRYPANOSOMIASIS</center>

We have had no opportunity for applying the new test in human cases of trypanosomiasis but a very large series of tests with the sera of rats, guinea pigs and rabbits infected with *Tr. equiperdum* conducted with the assistance of Miss Rule have shown that positive reactions do not occur with the new antigen although with antigens of the trypanosome strongly positive reactions are generally observed as early as one week after infection. The results of one such experiment are shown in Table I as an example of the whole series.

<center>LEPROSY</center>

Probably most interest is to be placed in the possibility of nonspecific reactions in leprosy. Undoubtedly the serum in this disease and particularly from cases of well advanced nodular leprosy may yield positive Wassermann reactions with most methods in common use but in a study of the new complement-fixation test in this disease by Denney and one of us[1] it was found

TABLE I

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN EXPERIMENTAL TRYPANOSOMIASIS*

RABBIT	C. L. ANTIGEN:**			SERUM DOSES:				TRYPANOSOME ANTIGEN:			SERUM DOSES:			
	.025	.012	.006	.003	.0015	.0008	Serum Contro	.025	.012	.006	.003	.0015	.0008	Serum Control
BEFORE INFECTION	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1 week	–	–	–	–	–	–	–	'***	3	1	–	–	–	–
2 weeks	–	–	–	–	–	–	–	4	4	4	4	4	2	–
3 weeks	–	–	–	–	–	–	–	4	4	4	4	4	4	–
4 weeks	–	–	–	–	–	–	–	4	4	4	2	1	–	–

*Infected with T. equiperdum.
**C. L. refers to the new Cholesterolized and Lecithinized Antigen.
***– = negative; 1 = +; 2 = ++; 3 = +++; 4 = ++++.

that falsely positive reactions did not occur. Positive reactions were, of course, observed, but in these there was either historical or clinical evidence of syphilis and we believe that the new test is not at all likely to yield nonspecific positive reactions in pure leprosy due primarily to the fact that the dose of antigen employed is 30 to 50 times less than its anticomplementary unit. With the old three antigen method apparently falsely positive reactions occurred in at least 7 to 8 per cent of apparently nonsyphilitic leprous patients in confirmation of numerous similar reports in the literature but these were not observed with the new method (Table II).

TABLE II

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN LEPROSY*

CASES OF LEPROSY	NUMBER TESTED	NEW METHOD		OLD METHOD	
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
With no Evidences of Syphilis	125	None	100%	7.2%	92.8%
With Evidences of Syphilis	27	100%	None	100%	None
Syphilis Suspected	7	None	100%	None	100%

*159 Cases: Nodular, 39; anesthetic, 32; mixed, 88.

TUBERCULOSIS

Recently Kilduffe[2] has shown that the new antigen does not yield falsely positive reactions in tuberculosis of nonsyphilitic individuals and a large series of tests conducted by one of us (J. A. K.) in cooperation with Dr. Callahan and to be separately reported upon, have yielded similar results. We are sure that the sera of nonsyphilitic tuberculous subjects do not yield positive reactions although several investigators claim that this may occur with the sera of some patients tested with the ordinary cholesterolized extracts.

PREGNANCY

The same is true with the sera of nonsyphilitic pregnant women tested at or near term. In the experience of Smith[3] the new test does not yield falsely positive reactions in this condition and the experience of one of us (J. A. K.) has been exactly similar. When positive reactions have occurred they have remained positive after delivery and have been observed only in

syphilitic women. In this connection it is to be borne in mind that pregnancy may stimulate or provoke latent syphilis into activity just as trauma, intercurrent disease, the administration of arsphenamine, etc., may prove provocative so that unexpected positive reactions may occur but when these are observed with the new method we believe that syphilis is present rather than that the reactions are falsely positive due to metabolic, toxic or other changes in the serum in late pregnancy.

DIABETES MELLITUS

Recently Rockwood and Sanford[4] have shown in an excellent serologic study of 501 cases of diabetes that falsely positive Wassermann reactions are of rare occurrence in this disease. Among these there were eight cases of probable coincidental syphilis with apparently falsely negative reactions with the sera of two by a modified Noguchi method and one by the Kolmer method;

TABLE III

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC CASES OF DIABETES MELLITUS

NO.	SEX	AGE (YRS.)	BLOOD SUGAR (MG. PER 100 C.C.	URINE SUGAR (PER CENT)	ACETONURIA	LIPEMIA	QUANTITATIVE COMPLEMENT FIXATION REACTION
1	M	43	378	6	+++	0	–*
2	F	24	170	2	0	Slight	–
3	M	60	246	2.5	+++	Marked	–
4	F	65	0	5	0	0	–
5	F	41	261	3.3	0	0	–
6	F	59	270	6	+	0	–
7	M	44	258	4.5	+	0	–
8	M	38	322	7	+++	Marked	–
9	F	56	0	4.2	0	0	–
10	F	58	300	5.6	0	0	–
11	F	60	0	2	0	0	–
12	M	50	374	7	++	Marked	–
13	F	57	0	3.5	0	0	–
14	M	60	272	3.3	+	Slight	–
15	F	32	332	7	++	Moderate	–
16	F	33	206	3	0	0	–
17	M	43	180	5-6	+++	–	–
18	M	68	308	6	++	Moderate	–
19	F	41	230	3-4	0	0	–
20	F	56	460**	4	0	0	–
21	F	29	236	5	+	–	–
22	F	42	240	4.5	0	Moderate	–
23	M	29	220	5	++	Marked	–
24	M	65	0	6	++	0	–
25	M	37	0	5.8	0	0	–
26	F	42	239	3.5	0	Moderate	–
27	F	32	172	2.4	0	0	–
28	M	37	186	3	–	0	–
29	F	70	0	4.5	–	0	–
30	F	47	178	1.5	–	0	–
31	M	43	210	4.8	++	Moderate	–
32	F	41	180	3	+	0	–
33	F	56	294	2	0	Marked	–
34	M	43	198	4.5	++	0	–
35	M	48	170	3	0	Moderate	–

*– = Negative; 0 = not determined.
** = Plasma CO_2 48 volume per cent.

five yielded positive reactions by the Kolmer technic but in two of these the evidences of syphilis were not conclusive. In 35 cases studied by us, several in coma, the reactions were negative as shown in Table III and it is our firm conviction that positive reactions by the new method in diabetes and especially repeated positive reactions are always due to coincident syphilitic infection.

MALARIA

Last year at the meeting of this society one of us[5] reported that in a study of the new test in malaria with sera kindly furnished by Dr. Bates of Ancon, Panama, and Dr. Caldwell of the Public Health Service in Houston, Texas, the reactions were uniformly negative with all but two negro patients

TABLE IV

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC CASES OF NEPHRITIS

NO.	SEX	AGE (YRS.)	ALBUMINURIA AND CASTS	TOTAL PHENOLSULPHONEPHTHALEIN RETURN IN 2 HOURS	BLOOD UREA (MG. PER 100 C.C.)	QUANTITATIVE COMPLEMENT FIXATION REACTION
1	F	34	+	5	145	—*
2	F	64	+++	10	52	—
3	F	45	+	35	25	—
4	F	19	+	0*	60	—
5	F	18	+	0	21	·
6	M	49	+	10	101	—
7	M	52	+++	0	67	—
8	M	61	+++	0	37	—
9	F	79	+	15	70	—
10	M	72	+	12	48	—
11	F	39	+++	65	15	—
12	M	42	+	0	150**	—
13	M	47	+++	0	55**	—
14	M	19	~	0	168***	—
15	M	51	++	0	170****	—
16	M	20	+	0	120	—
17	M	34	+	None	130*****	—

*0=not done; — =negative.
**=uremic coma.
***=uremic coma due to anuria.
****=blood creatinine 16.
*****=blood creatinine 4.

in whom the possibility of syphilis could not be definitely excluded. Since then tests with the sera of additional cases of tertian and aestivo-autumnal cases of malaria in individuals in whom the presence of syphilis could be excluded with a fair degree of accuracy, have yielded uniformly negative reactions and we are of the firm belief that the sera of nonsyphilitic malarial patients do not yield falsely positive reactions by this new method even when blood is drawn just before, during or after a paroxysm of chills and fever. The occurrence of positive reactions in malaria is presumptive evidence of syphilis requiring very careful clinical study.

NEPHRITIS, ICTERUS AND PNEUMONIA

From time to time investigators have reported that falsely positive Wassermann reactions may occur with the sera of nonsyphilitic individuals with

chronic nephritis with high retention, with icterus and pneumonia. In our experience reactions of this kind do not occur with the new method and we believe that they are relatively rare and exceptional with other methods in common use.

As shown in Table IV there is no tendency at all for falsely positive reactions in nephritis of nonsyphilitic individuals even when the blood is drawn during uremic coma. As shown in Table V falsely positive reactions do not occur with the sera of nonsyphilitic jaundiced individuals even when the sera are deeply colored with bile products which tends to increase their anticomplementary properties. The only case of croupous pneumonia in our series of cases which yielded a positive reaction was the serum of a syphilitic

TABLE V

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC
CASES OF JAUNDICE

NO.	SEX	AGE (YRS.)	JAUNDICE DUE TO:	QUANTITATIVE COMPLEMENT FIXATION RE-ACTION
1	F	46	Cholecystitis, etc.	—*
2	F	41	Cholecystitis, etc.	—
3	M	45	Cholecystitis, etc.	—
4	M	59	Carcinoma Pancreas	—
5	M	51	Carcinoma Pancreas	—
6	F	39	Cholelithiasis, etc.	—
7	F	50	Cholecystitis, etc.	—**
8	F	52	Biliary cirrhosis	—
9	M	62	Carcinoma Pancreas	—
10	M	38	Cholecystitis, etc.	—
11	F	47	Cholecystitis, etc.	—
12	F	38	Cholecystitis, etc.	—
13	M	50	Cholecystitis, etc.	—
14	F	50	Cholecystitis, etc.	—
15	F	43	Cholecystitis, etc.	—

*Negative.
**Serum discolored in dilutions as high as 1:40.

TABLE VI

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC
CASES OF CROUPOUS PNEUMONIA

NO.	SEX	AGE (YRS.)	TEMPERATURE (°F)	DAYS BEFORE CRISIS	QUANTITATIVE COMPLEMENT-FIXATION REACTION
1	M	20	101	5	—*
2	M	17	102.4	2	—
3	F	28	100.6	1	—
4	M	31	101.2	2	—
5	F	40	101.4	1	—
6	M	26	100	During	—
7	F	38	102.4	2	—
8	F	42	103	1	—
9	M	61	101.4	3	—
10	M	59	102	2	—
11	M	45	101.8	5	—
12	M	62	102	2	—
13	M	33	101.4	1	—

*Negative.

with a Type IV infection (Table VI). We feel absolutely certain that the sera of nonsyphilitic individuals in advanced nephritis, in jaundiced and in croupous pneumonia before or directly after the crisis, do not yield falsely positive reactions in this method when it is properly conducted.

SCARLET FEVER

Finally, a few words may be written regarding the Wassermann reaction in scarlet fever. As reported to this society last year there is no evidence at all of the occurrence of falsely positive reactions in this disease by the new method and we believe the same may be said of most other complement-fixation methods in common use today. We believe that it may be stated without reserve that the serum in all stages of scarlet fever does not yield falsely positive Wassermann reactions. In any large series of cases unexpectedly positive reactions may be observed in from 1 to 3 per cent of cases as is true in a Wassermann survey of any large number of sick or healthy individuals but we believe that when these occur with technically acceptable methods that the individuals are worthy of very careful study from the standpoint of the presence of latent congenital or acquired syphilis or of clinically atypical active syphilis.

Errors in the complement-fixation test for syphilis may be divisible therefore into two kinds, namely, (a) those due to errors in technic in both a falsely positive and a falsely negative way and (b) those due to biological factors beyond the control of the serologist. The percentage of the first group may be reduced to an almost negligible minimum by careful technic with special reference to the qualities of the antigen and in this society devoted to the principle of preserving and increasing the accuracy and practical value of medical laboratory procedures it is to be hoped that the members, individually and collectively, will exert themselves to foster a proper appreciation of the tremendous importance which the complement-fixation test can be made to possess for the diagnosis of syphilis and as a guide in its treatment when conducted by properly qualified serologists unwilling to be hurried in their work or to adopt anything that sacrifices the maximum degree of sensitiveness consistent with the specificity of the reactions. Under these conditions we believe that the chances of securing falsely positive reactions will be practically negligible and to the best of our knowledge positive reactions in the absence of syphilis will occur only in frambesia, or yaws, which is almost unknown in the United States. Individually and collectively it should be our aim to render available to the clinician a complement-fixation test for syphilis in which the occurrence of positive reactions including the unexpected weakly positive reactions, shall demand, not an apology or excuse on the part of the serologist, but a very careful reinvestigation of the history and clinical status of the patient for evidences of syphilis on the part of the clinician.

CONCLUSIONS

1. Falsely negative complement-fixation reactions may occur in cases of extremely latent syphilis because of insufficient amounts of "reagin" in the

blood and spinal fluid. The incidence of these may be reduced to a minimum by employing a test yielding reactions of as maximum a degree of sensitiveness as is consistent with specificity.

2. Falsely positive reactions are usually due to errors in technic and can be reduced to a minimum by confirming the conduct of the complement-fixation test for syphilis to competent serologists unwilling to sacrifice accuracy for mere speed in returning reports or for economy of time and materials.

3. The new complement-fixation test does not yield falsely positive reactions with the sera of rats, guinea pigs and rabbits with acute and chronic trypanosomiasis.

4. The new complement-fixation test for syphilis does not appear to yield falsely positive reactions with the sera of *nonsyphilitic* lepers or with the sera of *nonsyphilitic* individuals with acute and chronic tuberculosis, acute and chronic malaria, in advanced pregnancy, advanced diabetes, advanced neph ritis, acute pneumonia, acute scarlet fever or with jaundice due to nonsyphilitic involvement of the liver and its bile ducts.

REFERENCES

[1] Kolmer, J. A., and Denney, O. E.: The Wassermann Reaction in Leprosy with Special Reference to the New Complement-Fixation Technic, Arch. Dermat. and Syph., 1923, viii, 63.
[2] Kilduffe, R. A.: Concerning the Cross-Fixation of Syphilitic Antigen by Tuberculous Sera, Jour. Lab. and Clin. Med., 1923, ix, 124.
[3] Smits, F. C.: The Syphilis Complement-Fixation Reaction in Pregnancy with Special Reference to the Kolmer Reaction, Amer. Jour. Syph., 1922, vi, 705.
[4] Rockwood, R., and Sanford, A. H.: The Wassermann Reaction in Diabetes, Amer. Jour. of Syph., 1923, vii, 679.
[5] Kolmer, J. A.: The Specificity and Sensitiveness of the Author's New Complement-Fixation Test for Syphilis, Jour. Lab. and Clin. Med., 1924, ix, 468.

SOME PHYSICOCHEMICAL ASPECTS OF HEMOLYSIS*

By EDWARD C. MASON, M.D., DETROIT, MICH.,† AND REED ROCKWOOD, M.D., ROCHESTER, MINN.

INTRODUCTION

STUDIES in hemolysis have revealed the fact that salt solutions which prevent hemolysis, do so in concentrations having very nearly the same osmotic pressure; therefore, the theory has been advanced that the red corpuscle is enclosed in a semipermeable membrane, which swells or shrinks in response to the lowering or increasing of the osmotic pressure of the mediums surrounding the cell. Such a theory necessitates the assumption that the

hemoglobin is retained within the cell by a limiting membrane which, when taxed to the point of breaking, ruptures and allows the discharge of hemoglobin from the cell.

With more evidence at hand, one is inclined to question whether osmotic pressure plays any part in the process of hemolysis, for it is a well-established fact that isosmotic solutions of all salts and nonelectrolytes are not equally effective in preventing hemolysis. Neither can one explain the hemolysis by acids, alkalies, saponin, sodium oleate, bile salts, etc., on the basis of osmotic pressure changes, nor can the protective action of serum in the presence of various hemolytic agents be explained on a basis of altered osmotic pressure.

EXPERIMENTAL DATA

That the limiting surface of the red blood cell does not act as a retaining membrane is supported by observations such as are recorded in Fig. 1, a photograph of red cells which have been sectioned in various planes. Two hemolyzed "ghosts" are shown in the lower left corner for comparison. The photograph was taken ten hours after the cells were cut, and it will be ob-
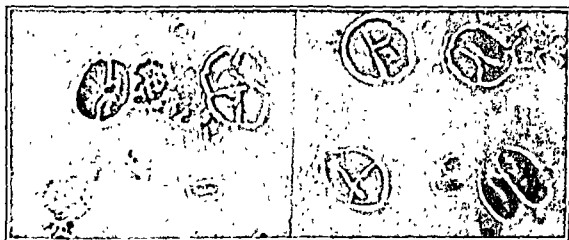

FIG. 1.

served that the stroma still retained the hemoglobin. Rollet observed that the cells could be mechanically injured without releasing the hemoglobin from its combination with the stroma and this observation has since been confirmed by others. Our method for sectioning the cells has been to grind them with washed sand in physiologic salt solution and float the cells from the mixture.

The various reactions of the red corpuscle warrants the conclusion that it is, essentially, a mass of colloidal components; the chief colloids, from the standpoint of hemolysis, being the stroma and the hemoglobin. Apparently the stroma-hemoglobin union is of a physicochemical nature and, judging from all the available evidence, has the characteristics of certain absorption phenomena. It is possible to use vehicles, other than stroma, to combine with the hemoglobin and the resulting combination behaves, in many respects, like the red corpuscle.

COMBINATION OF HEMOGLOBIN AND KAOLIN

If a solution of hemoglobin be filtered, it will pass through the filter paper unchanged; also, if it be shaken with kaolin and again filtered, it will

and is not absolute, but is the colorimeter reading obtained by using the least colored filtrate as the standard of comparison. The standard being practically colorless was given the value of 5. The precipitate remaining on the filter paper retained varying amounts of hemoglobin, the maximum, naturally, being in the one yielding the least colored filtrate. The precipitates were "hemolyzed" by treating each with an equal volume of concentrated solution of secondary sodium phosphate. The dotted line in Fig. 2 illustrates the results.

THE RELATION OF HYDROGEN-ION CONCENTRATION TO THE FRAGILITY OF RED CELLS

Believing that the stroma-hemoglobin combination of the red cell is, to a certain degree, similar to the kaolin-hemoglobin combination, we tested the response of the red cell to various hydrogen-ion concentrations. Our results are expressed in Fig. 3. Each of the eight tubes contained 1 c.c. of washed

A—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, 4.5 c.c. water, 32 hours.
B—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, 3.5 c.c. water, 20 hours.
C—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, 2 c.c. water, 17 hours.
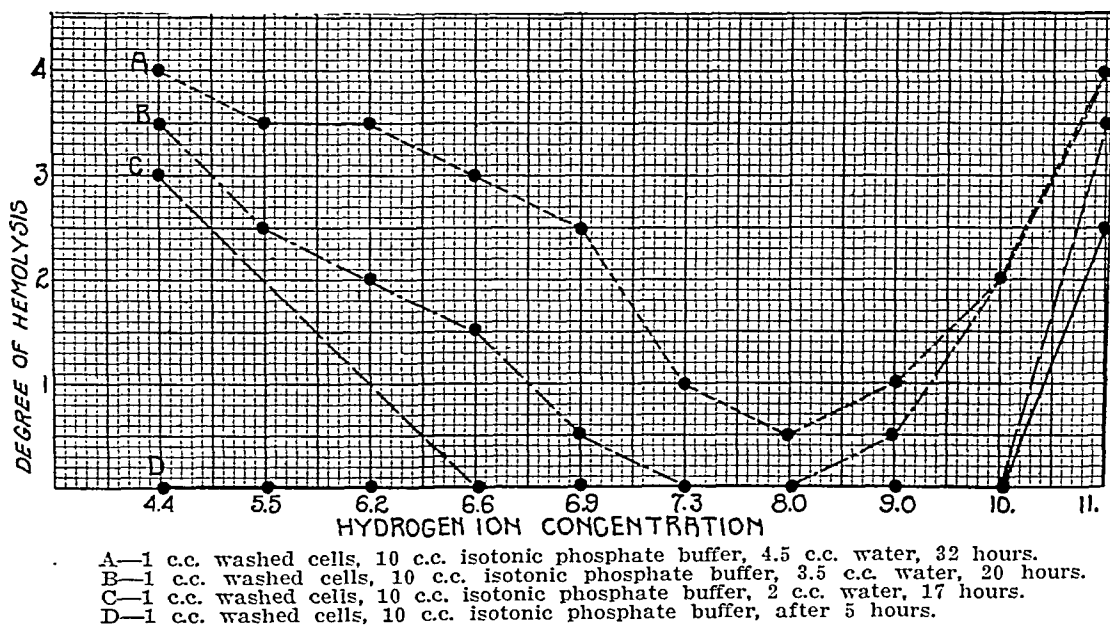D—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, after 5 hours.

Fig. 4.

cells and to each was added 10 c.c. of phosphate buffered solution made isotonic by sodium chloride. No hemolysis resulted in the first eighteen hours, at the end of which time 5 c.c. of distilled water was added to each tube, colorimetric readings were made and the results obtained gave the curve shown in Fig. 3. It will be observed that the curve is quite similar to that obtained from the kaolin-hemoglobin combination and differs mainly in that the kaolin-hemoglobin combination was most stable on the acid side, while the stroma-hemoglobin was most stable in the middle and toward the alkaline side. Both show a zone of greatest stability, the exact range of which has not been determined.

SALT PROTECTION AGAINST ACID AND ALKALI HEMOLYSIS

The results, expressed in Fig. 3, show salt protection against acid and alkali hemolysis, for, as previously stated, there was no hemolysis within the

first eighteen hours; however, when 5 c.c. of distilled water was added to each tube, there was definite hemolysis on both sides of the optimal hydrogen-ion concentration. Fig. 4 is presented to show somewhat the same condition but
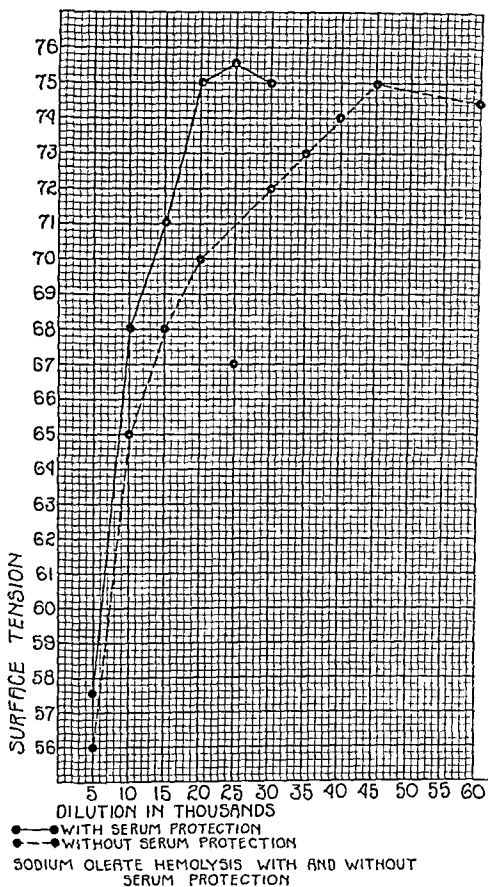

WITH SERUM PROTECTION

WITHOUT SERUM PROTECTION

SODIUM OLEATE HEMOLYSIS WITH AND WITHOUT
SERUM PROTECTION

Fig. 5.

approached more gradually. Ten tubes were used, the range in pH being from 4.4 to 11 and the degree of hemolysis expressed in terms of 4, complete hemolysis being graded as 4. Each tube contained 1 c.c. of washed cells in 10 c.c. of isotonic phosphate buffer solution. Line D ex-

presses the results at the end of five hours, there being no hemolysis except in the tube with pH of 11. Water was gradually added, as expressed by C, B and A, and the resulting hemolysis charted. It will be observed that the tubes, more acid or more alkaline than the optimal range, became partially and even completely hemolyzed before any hemolysis appeared in the range of optimal hydrogen-ion concentration; also, since such hemolysis appeared only with dilution, it must be concluded that the salt concentration prevented acid and alkaline hemolysis.

SURFACE TENSION AND HEMOLYSIS

Many of the hemolytic agents have no chemical properties in common. However, lowering of surface tension is one striking physical property common to the most active. We have made determinations on surface tension and its relation to hemolysis, and an outline of our results is presented in Fig. 5. The apparatus used was that designed by DuNoüy.[2] The readings presented have not been corrected to read in dynes; they were recorded directly from the dial and should be accurate enough for comparison. The surface tension of sodium oleate was studied without and with the presence of serum protection. A series of twelve tubes was used, the first tube containing sodium oleate, in a concentration of 1-5000; the second, 1-10,000 and the dilution increased in each tube by 5000 parts of physiologic salt solution, until the last tube contained sodium oleate in a concentration of 1-60,000 of sodium chloride solution. It was observed that sodium oleate was active in producing hemolysis in concentration of 1-40,000 to 1-50,000. Surface tension determinations were made on the series of twelve tubes and found to range from 56 in the tube containing sodium oleate 1-5000 to 80 in the tube containing sodium oleate 1-60,000, the reading for salt solution being 91 (Fig. 5). It will be noted that, without serum protection, it required nine tubes to reach a surface tension of 75 and the dilution in such a tube was 1-45,000 but, with serum protection, only four tubes were needed to reach a surface tension of 75, and such a tube contained sodium oleate 1-20,000. These results are unexpected, for one would not anticipate that a substance, with as low surface tension as blood serum, would increase the surface tension of another substance. The results may be due to a direct combination of serum and sodium oleate.

HEMOLYSIS BY HYPOTONIC SALT SOLUTIONS

The surface tension difference, between a salt solution which is isotonic and one that is hypotonic, is entirely too slight to be considered as an important factor in hemolysis by hypotonic salt solutions. In hemolysis by hypotonic salt solutions, swelling preceded or accompanied the hemolysis and it is usually implied that, when swelling is sufficient to rupture the limiting ''membrane,'' the hemoglobin is discharged. We have demonstrated that hemolysis may or may not be accompanied by increased volume of the red cell and, also, that cutting of the cell does not cause the discharge of hemoglobin.

DISCUSSION

We have prepared the model "red cells" which differ from those previously prepared in that we have used hemoglobin as one of the constituents. We have also been able to use various vehicles, other than stroma, to combine with the hemoglobin and have observed that such combinations were most effective over a zone near the neutral point.

Because of the lack of a better term to express the forces operating to combine these constituents, we have been compelled to use the much misused term "adsorption." Mathews has recently written a most illuminating paper on the subject of adsorption, in which he presents a plea for a less loose use of the term.

The following, under the section on "Chemical Adsorption of Substances and Solution by Solids," has a most direct bearing on the present discussion: "Under this heading are found most of those cases of adsorption with which we are familiar, such as decolorization by the use of charcoal; staining and dyeing; tanning; the precipitation of proteins; precipitation of one colloid by another; capillary analysis by means of filter paper. All of these are cases of adsorption, for the reason that the adsorbing substance is of a coarseness at least equal to that of the smallest colloidal particle in diameter. But this, however, does not alter their chemical powers, and in most or all of these adsorptions there can be little doubt that chemical substitutions rather than forces of surface tension, or physical forces, are involved."

It is evident that our kaolin-hemoglobin and stroma-hemoglobin systems belong to this comprehensive group; however, it seems impossible for us to rule out, as Mathews does, the influence of surface tension, for lowering of surface tension is one of the most effective methods of breaking the stroma-hemoglobin union.

We wish to agree with Mathews when he states that "surface tension is but one of the indirect results of the force of cohesion" and, also, that "cohesion is one of the forces of attraction of nature; surface tension is not." Therefore, since lowering of surface tension so closely parallels the breaking up of the stroma-hemoglobin adsorption, it appears wise to analyze the term "cohesion." Mathews states that "Chemical affinity is either electrical or magnetic, and probably the former; cohesion is neither of these. Chemical affinity appears to vary directly as the number of valences and, inversely, as the atomic weight, whereas, cohesion varied directly as the molecular weight, that is directly as the gravitational mass. Cohesion involves both gravitational affinity and chemical affinity." From the last sentence, it appears that cohesion is a much broader term than chemical affinity and, since chemical affinity does not comprehend surface tension changes, it becomes necessary for us to use the term cohesion. However, the term cohesion should be restricted to express attraction within a homogeneous system, and the term adhesion to express attraction within a heterogeneous system.

We have shown that the stability of the kaolin-hemoglobin and stroma-hemoglobin systems were disturbed with the addition of acid or alkali and have also shown salt protection against acid-alkali hemolysis. These facts,

taken together with the fact that the two amphoteric colloids, forming such systems, combine only within a zone close to the neutral point, suggests a possible relation to the observation made by Bredig. He observed that at the boundary of a particle in contact with water there is developed a force of surface tension. When the particle is electrically charged the surface tension is diminished, but, when the particle is discharged, the surface tension reaches a maximum. Therefore, at the isoelectric point, where the charge on the particle is nil, the surface tension is a maximal and such a point favors combination of the particles.

A study of blood coagulation and hemolysis furnishes some most interesting points of comparison. It will be observed that all agents, which tend to change the red cell from a state of (1) small mass, (2) low hydration, capacity, (3) maximal cohesion and, (4) relative electrical neutrality, (all meaning essentially the same), to a state of (1) larger mass, (2) increased hydration capacity, (3) decreased cohesion and (4) away from the isoelectric point, do, to the same degree, promote hemolysis.

Coagulation of blood in some respects, on the other hand, appears to be just the reverse process of hemolysis. There is a rapid change, in blood coagulation, from a mass of high hydration capacity to one of low, and such process is accompanied by a marked change in cohesion, as shown by the enormous increase in surface tension. In regard to the isoelectric point during coagulation, it is interesting to note that the substances which accelerate coagulation are for the most part electronegative, while autolyzed tissue, which really represents tissue decomposed in relative acid condition, will retard coagulation.

CONCLUSIONS

1. The red blood cells may be cut without the subsequent discharge of hemoglobin.

2. Hemoglobin may be made to combine with vehicles other than stroma; such combination with kaolin is most effective over a definite zone of hydrogen-ion concentration.

3. The fragility of the stroma hemoglobin combination varies greatly with the hydrogen-ion concentration, the union being most stable in the zone of pH 7.3-8.0.

4. Charts are presented to show a definite salt protection against acid and alkali hemolysis.

5. Serum protection against hemolysis by sodium oleate parallels the increase of surface tension produced by adding the serum.

6. The stroma hemoglobin union is of a physicochemical nature and has the characteristics of adsorption phenomena.

REFERENCES

[1] DuNoüy, P. L.: A New Apparatus for Measuring Surface Tension. Jour. Gen. Physiol., 1919, i, 521-524.

[2] Mathews, A. P.: Adsorption. Physiol. Reviews, 1921, i, 553-557.

[3] Michaelis, L.: Die Wasserstoffionen-Konzentration, Berlin, 1914, pp. 49-50.

[4] Rollett, A.: Elektrische und thermische Einwirkungen auf das Blut und die Structure der rothen Blutkörperchen. Arch. f. Physiol., 1900, lxxxii, 199-267.

PHYSICOCHEMICAL ASPECTS OF HEMOLYSIS. II. AN ULTRA-MICROSCOPIC STUDY OF HEMOLYSIS*

By Reed Rockwood, M.D., Rochester, Minnesota
(Fellow in Medicine, Mayo Foundation)

IN connection with other studies[9] that have been made on hemolysis, the process of hemolysis was studied by means of the ultramicroscope, with special reference to the morphologic changes produced by different concentrations of different hemolytic agents.

Bechhold, Kraus, Salen and Hattori, working in Bechhold's laboratory, have reported observations on hemolysis with the use of the ultramicroscope. By the use of enzymes, various lipoid solvents, and protein precipitants, they have shown which portions of the cell are composed of protein, and which of lipoid, and they have demonstrated the various ultramicroscopic patterns formed when one or the other constituent is altered. Aside from this, their main interest has been in the effect of solutions of mercury, of which weak concentrations hemolyze the cell, and stronger concentrations precipitate the cell proteins.

As a result of these extensive observations, Bechhold believes that the *hemolysis is due to changes in the stroma. Any condition which causes the* stroma protein, cholesterin or lecithin to be separated will cause the release of the hemoglobin from combination. It is not necessary for all three constituents to be affected since, for example, a change in the lipoids alone may occur which will be effective in causing hemolysis.

METHOD OF STUDY

A Siedentopf cardioid condenser was used with a quartz object chamber. A 1000-watt Mazda lamp in a stereopticon served as the source of illumination. It was found that a water filter was inadequate to filter out the heat rays which would change to some extent the cell structure; a gold glass filter, which filters out 95 per cent of the heat rays was therefore substituted. Solutions of different percentages of the hemolytic agent were made, and one drop of solution was mixed in the chamber with one drop of freshly drawn human blood (taken from the same individual in each instance) and immediately examined.

In taking the photographs, a time exposure was found to be necessary. The Brownian movement in the cells often obscures the details of cell structure. For this reason a series of drawings has been added to show finer details. Because of the difficulty in keeping the illumination passing through

the camera constant from day to day, the intensity of the light reflected from the cells appears to vary from photograph to photograph. The prints have therefore been retouched to bring out the proper contrast. In general, it may be said that there are only two degrees of intensity of light, one in the hemolyzed cell, and the other in the cell not hemolyzed. Certain exceptions to this occur in hemolysis by distilled water and in the reversal of hemolysis. The hemokonia, because of their small size and rapid motion, do not photograph. The difference in brightness between the hemolyzed "ghosts" and their background is very slight. It is very difficult to adjust the illumination so as to impress them on the plate. Therefore, the number seen in the photographs is usually considerably less than the number actually present. Photographs were taken of all the dilutions presented in this paper. Only a few are shown here as examples.

In the study of the cells a 1.8 mm. objective was used with a 20x Huyghenean eyepiece, giving a magnification of about 1900 diameters.

THE NORMAL ERYTHROCYTE

Normal human erythrocytes are well shown in Fig. 1. As seen from above, the corpuscle appears as a brightly illuminated ring surrounding a dark area, through which at times a little light is reflected from the lowest portion of the cell. This gives a false idea of the cell structure, and is due to the fact that the light is only reflected from the surface as it comes from the side. As the cells roll over and over in the field, however, or, as they are seen in rouleau formation, it is evident that all parts of the cell surface in turn will give a similar bright reflection.

THE TYPES OF ERYTHROCYTES SEEN IN HEMOLYSIS

Only a limited number of cell types are observed under different conditions (Figs. 2, 3, 4 and 5). Swollen forms, similar to the normal, but much enlarged, are sometimes seen (Fig. 2). The cell which has been hemolyzed appears as a faint gray-white ring standing out but slightly from the dark background, and smaller than the original cell from which it came (Fig. 3).

Certain cell forms have long been known to microscopists and called "crenated." It seems to me, after a careful study of these forms, that two groups have been included here: one, a true crenation, and the other, consisting of various degrees of stromatolysis. The truly crenated form is apparently a shriveling of the cell without cell destruction, and is probably due to some alteration in the water balance between the cell and its surrounding medium. The stromatolytic form is due to an actual destruction of the cell, particularly at its surface. Both forms may be combined to a greater or less extent, or a cell may first be crenated, and later stromatolysis occur. These forms may also be observed in ordinary microscopic fields from the same preparation, although their recognition is much facilitated by the intense illumination of the cell surface by the ultramicroscope. I cannot always distinguish between these different forms in the case of the individual cell, but by a study of several fields in the same preparation,
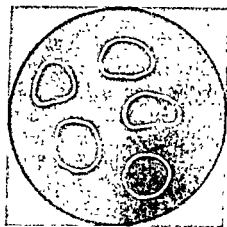
Fig. 1—Normal erythrocytes.

Fig. 5.—Stromatolytic forms, showing thickening of the outer rim with nodular distorted appearance.

Fig. 4.—Crenated forms showing scallopng of the cell margin.

Fig. 3.—Hemolyzed cells.

Fig. 2.—Swollen cells similar to the normal but much enlarged.

observing cells as they roll over and over in the field, and watching the progress of hemolysis and stromatolysis, or by using different concentrations of the same reagent, I can usually decide, in a given preparation, which process predominates.

The crenation (Fig. 4) starts as a scalloping of the cell margin. By focusing it may be seen, however, that the surface of the cell is intact, and that the two borders of the ring of light surrounding the cell are nearly parallel, as in the normal cell. As the shriveling of the cell continues, small ''chrysanthemum'' and ''hydrangea blossom'' forms are produced, the original source of which is impossible to determine, unless the process has been followed, or unless distinguishing characteristics are present.

On the other hand, in the stromatolytic forms (Fig. 5), a thickening of the outer rim may produce a nodular distorted appearance. It suggests an aggregation of the colloids of the surface into larger particles. Then pieces of the cell surface may be distinctly seen to break off and float away into the solution, leaving gaps in the surface layer which persist even after care-
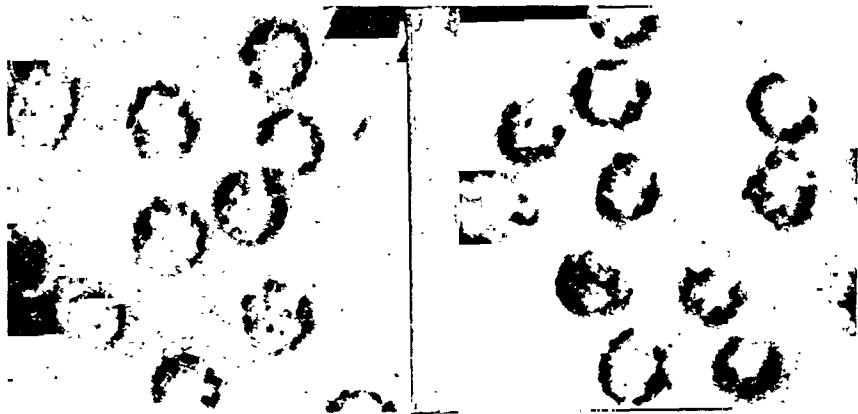

Fig. 6.—Stromatolysis without hemolysis.

ful focusing. This process may also result in chrysanthemum or hydrangea blossom forms, similar to those of the crenated cell. In certain cases the surface breaks down, forming long, trailing, gray-white streamers with brighter spots beaded along their course, giving a Medusa-like appearance. These forms are particularly observed in cells in which stromatolysis occurs after standing for a few hours at room temperature. Bechhold has noticed these forms as a result of treatment with mercury.

HEMOKONIA-LIKE BODIES

Numerous brilliant granules, corresponding to the descriptions usually given for hemokonia, are seen in all of these preparations of the solutions of the hemolytic agent mixed with blood. The pieces of cell broken off in the process of stromatolysis cannot be distinguished with the microscope from those originally present in the serum. Porter, in making microscopic studies of autolyzing blood, also observed the formation of hemokonia-like bodies from these stromatolyzing forms. If the corpuscles are examined, diluted with serum in the chamber, their number is relatively small. If

diluted with isotonic salt solution, there is a very distinct increase, and this also confirms the work of Hamburger, who found that cells which had been washed in isotonic salt solution or Ringer's solution revealed the presence of lecithin by chemical tests in the solution, and were less resistant to hemolysis. He found also that this lack of resistance could be done away with by the addition of more lecithin to the cells. The descriptions which have been given of hemokonia indicate a lecithin-like substance. These bodies are smaller and appear to be different from those described by Rous and Robertson as "fragmentation" forms. It should be noted that these particles are seen in shed blood after treatment with various reagents, and aside from their morphology there is nothing to identify them with the hemokonia seen in the circulating blood.

If other agents are added which cause distinct stromatolysis, the number of hemokonia-like bodies is increased enormously, and this increase appears to be roughly parallel to the amount of stromatolysis which is observed. It will be noted that there is an increase in the number of hemokonia-like bodies before any definite stromatolysis can be observed by the ultramicroscope, as in the case of isotonic salt solution.

CHANGES PRODUCED BY ALLOWING CELLS TO STAND

If corpuscles in oxalated blood are allowed to stand, even in the ice box, changes begin to occur relatively early. Within five or six hours distinct stromatolysis is evident, and numerous particles may be seen to be breaking *off from the surface of the cells, even though they are standing* in their original serum. Some crenation may be present as well, but the main process is that of stromatolysis. Hemolysis, under these conditions, however, does not occur for some days. Thus, the cells in the preparation illustrated in Fig. 6 show a marked stromatolysis of the cell surface, but hemolysis did not commence until two days after the photograph was taken. This cellular degeneration is not constant and occurs faster in some specimens of blood than in others. It seems to be favored by centrifuging, or by washing the cells in isotonic salt solution.

These observations show the relatively small part which the boundary between the cell and the plasma can play in hemolysis, since its marked alteration does not result with the release of the hemoglobin from the stroma. It has been shown[9] that the cell could be cut in pieces without the loss of hemoglobin. This boundary or apparent contact surface of the cell must be distinguished from the actual contact surface of the hemoglobin-stroma union and its surrounding intracellular medium.

HEMOLYSIS BY DISTILLED WATER

The actual process of hemolysis as seen under the ultramicroscope is substantially the same for all the hemolytic agents that have been studied. Two points are noticeable: the rather rapid destruction of the individual cell, which loses its hemoglobin in from three to five seconds, and the marked irregularity in the cell's resistance to hemolysis, many cells persisting for

a long time in the solution. This is particularly true with certain hemolytic agents. As the cell hemolyzes, it uniformly fades from the bright rim of light seen in the normal cell to the faint gray-white rim which outlines the disk of the ghost. At the same time it shrinks a little from its original size, and this process may continue for a short time after the rim has become completely pale.

In hemolysis by distilled water, there are one or two additional characteristics. The hemolysis is slower than the usual time of three to five seconds, and many intermediate gradations in brightness are visible between the primary and final forms. The cells are swollen and lose their biconcave appearance. There is no definite stromatolysis to be observed in the cells or ghosts, but there is a distinct increase in the number of hemokonia-like bodies in the field, particularly with the higher percentages of distilled water (60 to 70 per cent).
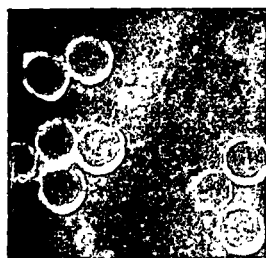
HEMOLYSIS BY ACID

In hemolysis by the addition of hydrochloric acid, there is also considerable agglutination which at times makes interpretation of the results difficult (Fig. 7). Hemolysis seems to depend not only on the concentration of the hemolytic agent, but also on the relative concentration of hemolytic agent and cells, and the time which is allowed to elapse. This is also characteristic of other hemolytic agents besides acid. Thus, with hydrochloric acid (N/500) with sodium chloride solution, hemolysis occurs in a few cells if not many are in the preparation, but not at all if there are quite a number in the field. From N/330 upward, agglutination begins to occur and this is more marked at about N/200. At N/166 and N/142, both hemolysis and agglutination are present. The agglutinated cells seem more resistant to hemolysis. With the higher concentration of acid, N/142, there also seems to be a little stromatolysis. This is independent of the hemolysis. That is, hemolysis may occur before stromatolysis or after, and the ghosts may be destroyed, in some cases after hemolysis.
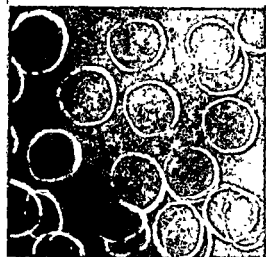
HEMOLYSIS BY ALKALI

By the use of sodium hydroxid, nothing especially characteristic is seen. There is a little stromatolysis visible in about N/142 dilution and the cells hemolyze as described.
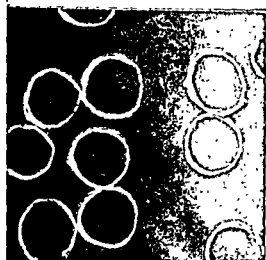
HEMOLYSIS BY SAPONIN

The hemolysis of individual cells in saponin (1/1000) is very rapid, but many cells are quite resistant. Most of the cells in 30 and 40 per cent dilutions of 1/1000 saponin hemolyze without any visible change in the cell surface, but the surface is almost immediately attacked, although at a slower rate, and glittering particles can be seen to become detached, giving typical hydrangea-blossom forms. These cells may or may not become hemolyzed. Four forms may be seen in the field at the same time in these concentrations: normal cells, normal ghosts, stromatolyzed cells, and stromatolyzed ghosts. The study shows the independence of the processes of hemolysis and stromatolysis.
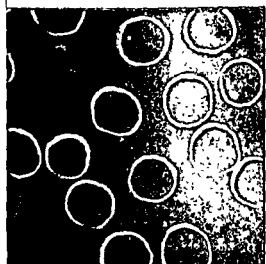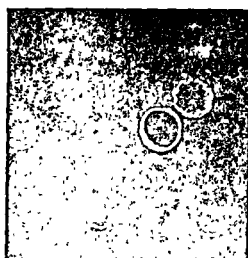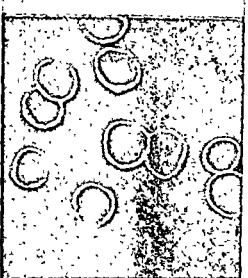
N/250

N/500

N/1000

0°

N/142

N/142

N/166
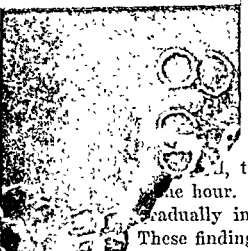
*Strength of hydrochloric acid.

Fig. 7.—Hemolysis by acid.

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HEMOLYSIS BY SODIUM OLEATE (1/1000)

Stromatolyzed forms are found very early in the series, but all seem to begin as crenation, although, later, particles can be distinctly seen to break off. There is a marked difference in the size of the cells, which seems to be
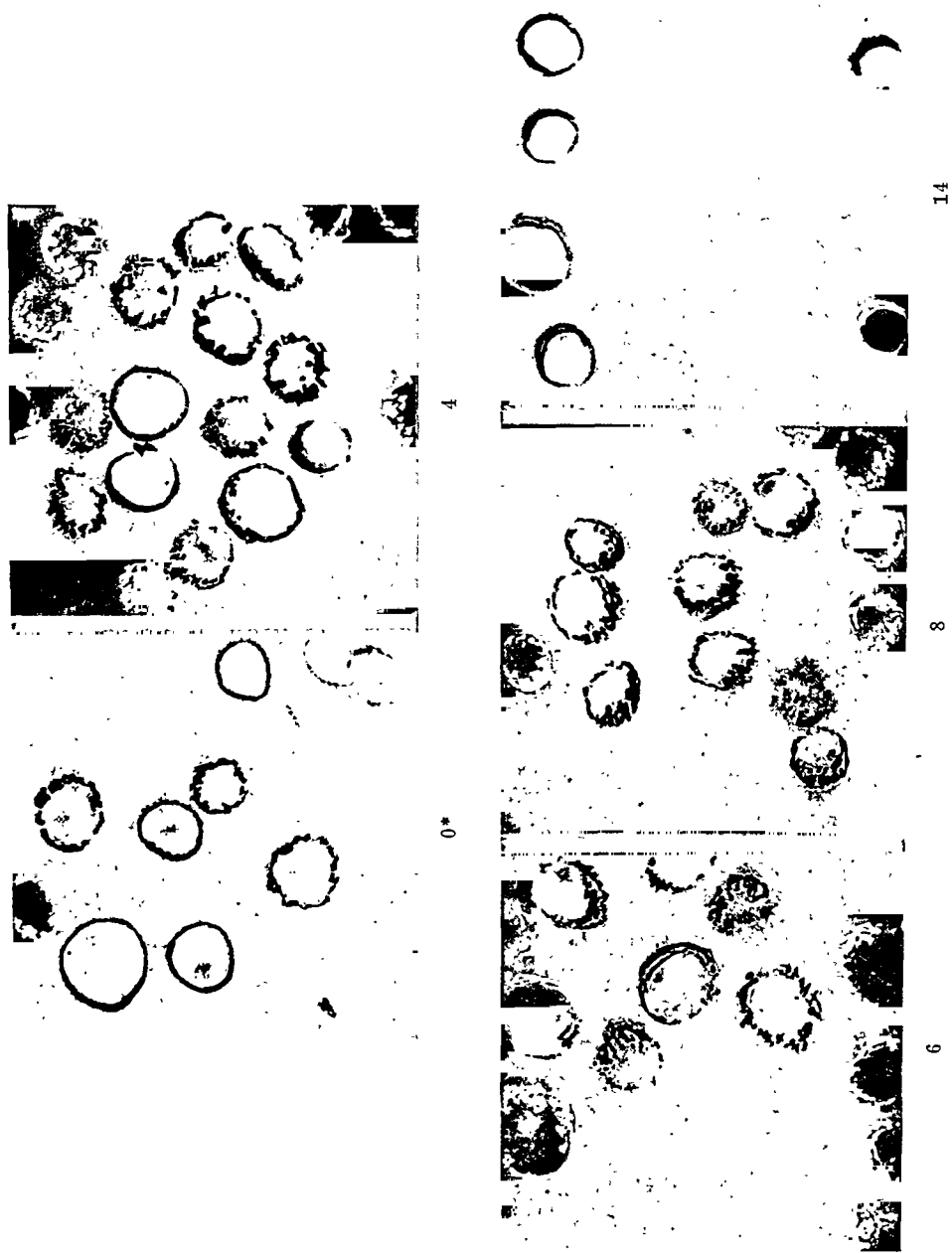
Fig. 8.—Hemolysis by autolysis and action of sodium chloride. *Percentage of 20 per cent sodium chloride.

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welling, and to a difference in resistance. Since normal, crenated, itolyzed forms are seen in the same field, the latter predominating, demonstrated that stromatolysis and crenation are not essen- and that the processes go along independently. In most ns to occur without swelling of the individual cell.

THE ACTION OF HYPERTONIC SALT SOLUTION

The action of hypertonic salt solution shows many curious phenomena which I am not able to explain. Crenation is greatest at from 2 to 6 per cent; there is some hemolysis at 6 per cent. Beyond this, crenation is slight and a large portion of the cells may even be swelled. Hemolysis also depends on the time of action since it becomes very marked in the 6 per cent solution if it is allowed to stand a number of minutes. Stromatolysis is also very definite at this concentration, and granules can be seen to break off singly and in groups. At 14 per cent there is a little crenation, although it appears in typical form in only a few cells. Hemolysis occurs, with and without crenation and stromatolysis, although principally without. The majority of the cells are not swollen, although a few in each field are markedly so. Distinct caput Medusa-like forms with waving arms, which may break off, are seen. These have been described by Bechhold, and I believe that they are merely atypical forms of stromatolysis.

HEMOLYSIS BY AUTOLYSIS AND HYPERTONIC SALT SOLUTION

By autolysis I mean the hemolysis and associated changes which take place in the red cell after standing from twelve to eighteen hours at room temperature. Simple autolysis causes extreme stromatolysis of the cell. Bacteria are seen at times. The hemolyzed stromas appear as usual, except that some may have brightly refractile granules still attached. Bechhold believes that these are inside the ghost, rather than on the surface. The granules throughout appear larger than those ordinarily seen. There is a tendency for a considerable number of the cells to appear swollen, but many are not. Some of the swollen cells show stromatolysis.

Autolysis may be prevented by the addition of from 2 to 4 to 8 per cent of hypertonic salt solution. If a higher concentration is used, hemolysis occurs again, probably due in part to the hemolytic action of the larger amounts of salt (Fig. 8). The mechanism of this action will be discussed in a later paper. In the high concentration of sodium chloride (10, 12, or 14 per cent), hemolysis has evidently occurred so rapidly that little time has been given for stromatolysis, and the cells have been protected in some manner so that stromatolysis does not occur to any extent, even after standing so many hours.

BIOLOGIC HEMOLYSIS

There is no definite morphologic change in the cells in hemolysis by complement and amboceptor. The surrounding rim fades out without any change in cell volume. Apparently this type of hemolysis releases the hemoglobin with less distinct disturbance of the cell than any of the others studied.

HEMOLYSIS BY HEAT

In order to observe the effect of heat on the red blood cell, the gold glass filter is removed from the source of illumination for one hour. Within a few minutes, a few cells hemolyze, and the number gradually increases. There is some stromatolysis, but this is relatively slight. These findings serve

DISCUSSION

At the last International Physiological Congress at Edinburgh, Brinkman reported further results on the reversal of hemolysis. Through the kindness of Brinkman and Szent Györgyi, I have received a copy of their paper in advance of publication. They, too, have repeated this older work and have extended it to include hemolysis by freezing and thawing, and hemolysis by linolenic acid. With this latter substance they have secured their most complete reversal. They have made careful studies of the refractive index of hemolyzed and reversed blood, and have clearly shown that in the course of hemolysis, all the hemoglobin leaves the cell, while with reversion it comes back to an extent of not less than 60 per cent. This definitely proves the actual fact of reversion and disposes of arguments based on optical effects.

I have also confirmed this work with linolenic acid. Under the ultramicroscope it can be seen that the cells are restored without crenation, so that they cannot be distinguished from the normal cells as shown in Fig. 1. Here again, there is a difference in the brightness of the various cells, due to incomplete reversal. Every cell seems to brighten in its outline, indicating that it takes up some hemoglobin, but certain cells resume the original degree of brilliancy. No stromatolysis is observed at any time in the stroma. No definite change in volume is observed during hemolysis by linolenic acid and reversion. Hemolysis by this substance approaches more closely morphologically that by complement and amboceptor than any which I have studied.

Other facts, also, point to the existence of reversed hemolysis. Thus, von Krogh has shown that hemolysis by sodium hydroxide can be reversed in part by the addition of the proper amount of hydrochloric acid. Fischer has made model erythrocytes of fibrin and carmin, and has shown that such mixtures are "hemolyzed" by distilled water, acid, alkali, urea, ammonia salts, autolysis, and heat, in a manner similar to the hemolysis of the true cell. Mason and Rockwood have shown in a previous paper that hemoglobin and kaolin, and hemoglobin and agar form reversible compounds which behave, in some respects, as do the true erythrocytes.

CONCLUSIONS

1. The ultramicroscope shows that many forms of cells which are commonly called "crenated" are, in reality, various forms of stromatolysis.

2. Stromatolysis and hemolysis are apparently independent processes, and stromatolysis is more marked with certain hemolytic agents at certain concentrations than at others.

3. The process of the formation of hemokonia-like bodies can be observed.

4. Hypertonic salt solution causes crenation in low dilutions, but causes stromatolysis, cell swelling and hemolysis as the concentration is increased.

5. Hemolysis by autolysis may be prevented by certain concentrations of hypertonic salt solutions.

6. The apparent cell surface plays a relatively small part in the process of hemolysis since it may be profoundly altered by stromatolysis at least two days before hemolysis occurs.

7. The fact that a true reversal of hemolysis may occur under the proper conditions may be confirmed by the ultramicroscope.

BIBLIOGRAPHY

[1]Adair, G. S., Barcroft, J., and Bock, A. V.: The Identity of Hemoglobin in Human Beings, Jour. Physiol., 1921, lv, 332-338.

[2]Bechhold, H.: Bau der roten Blutkorperchen und Hämolyse. München. med. Wchnschr., 1921, lxviii, 127-130.

[3]Bechhold, H., and Kraus, W.: Kolloidstudien über den Bau der roten Blutkörperchen und über Hämolyse, Biochem. Ztschr., 1920, cix, 226-235.

[4]Brinkman, H., and von Szent Györgyi, A.: The Reversion of Hemolysis, Jour. Physiol., 1923, lviii, 204-208.

[5]Fischer, M. H.: Edema and Nephritis, 2 ed, New York, Wiley, 1915, p. 364.

[6]Hamburger, H. J.: The Increasing Significance of Permeability; Problems for the Biological and Medical Sciences, Bull. Johns Hopkins Hosp., 1923, xxxiv, 266-274.

[7]Hattori, K.: Kolloidstudien über den Bau der roten Blutkorperchen und über Hämolyse. III. Ultramikroskopische Untersuchungen an Lipoide, Biochem. Ztschr., 1921, cxix, 45-64.

[8]Koeppe, H.: Über das Lackfarbenwerden der roten Blutscheiben. Dritte Mitteilung. Arch. f. d. ges. Physiol., 1905, cvii, 183-186.

[9]Mason, E. C., and Rockwood, R.: Some Physico-Chemical Aspects of Hemolysis, JOUR. LAB. AND CLIN. MED., 1924, x, p. 10.

[10]Porter, F.: Observations on Blood Films, with Special Reference to the Presence of Hemoconin, Brit. Med. Jour., 1907, ii, 1773-1774.

[11]Rous, P., and Robertson, O. H.: The Normal Fate of Erythrocytes, Jour. Exper. Med., 1917, xxv, 651-673.

[12]Salén, E.: Kolloidstudien über den Bau der roten Blutkörperchen und über Hämolyse. II. Ultramikroskopische Untersuchungen an Stromata, Biochem. Ztschr., 1920, cx, 176-180.

[13]Schwalbe: Quoted by Ruszynák, S.: Beiträge zu einer Chemie der Hämolyse, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1911, viii, 421-432.

[14]Siedentopf, H.; Über einen neuen Fortschritt in der Ultramikroscopie, Ber. d. deutsch. physik. Gesellsch., 1910, viii, 6-47.

[15]Spiro: Quoted by Ruszynák: Beiträge zu einer Chemie der Hämolyse, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1911, viii, 421-432.

[16]von Krogh, M. L.: Ueber die Reversibilität der Hämolyse, Biochem. Ztschr., 1909, xxii, 345-351.

THE IMPORTANCE OF ABSORBING NATURAL ANTISHEEP AMBOCEPTOR FROM SERUMS TO BE USED IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By RUTH GILBERT, A.M., M.D., AND M. J. WEMPLE, A.B.

THERE is considerable divergence in the results obtained by different investigators as regards the influence of natural antisheep amboceptor in human serums upon the complement-fixation test. Some workers find that the effect of natural amboceptor is so slight that it can be considered negligible while others believe that it introduces a definite source of error.

Those who hold the latter view have sought to eliminate this source of error either by utilizing the natural amboceptor in each serum or by absorbing it with sheep cells. The question of the practical necessity of removing natural amboceptor in this way has recently been studied in this laboratory.

To begin with, a satisfactory method, partly adopted from methods described by others, for absorbing natural amboceptor from human serums was developed. It is effective in removing amboceptor, does not cause the serum to become anticomplementary and is less time-consuming than some of the methods used in other laboratories. Briefly, it is carried out as follows:

To 1 c.c. of serum and blood cells (that is, blood poured from the clot before centrifugalization) 0.08 c.c. of washed packed sheep cells are added and the mixture is allowed to stand ten minutes at 3-6° C. The sheep cells and blood must be cold before mixing in order to prevent hemolysis due to the complement in the patient's blood. The serum recovered after centrifugalization is inactivated and is then ready for use in the complement-fixation test.

In a series of over 6000 serums submitted for the routine complement-fixation test for syphilis at this laboratory, only 730, or approximately 12 per

SUMMARY OF RESULTS OBTAINED IN COMPLEMENT-FIXATION TESTS BEFORE AND AFTER ABSORPTION OF NATURAL ANTISHEEP AMBOCEPTOR

Part I Readings Made Immediately After Hemolysis of Serum and Antigen Controls.
Part II Readings Made After the Tests Had Stood Overnight in the Cold Room.

	NUMBER OF REACTING SERUMS TESTED BEFORE AND AFTER ABSORPTION	NUMBER OF SERUMS SHOWING NO CHANGE IN DEGREE OF FIXATION		SERUMS SHOWING INCREASED DEGREE OF FIXATION AFTER ABSORPTION						
				TOTAL NUMBER OF SERUMS		NO FIXATION TO SLIGHT FIXATION		PARTIAL TO COMPLETE FIXATION		SLIGHTLY INCREASED DEGREE OF FIXATION
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO. PER CENT
Part I	148	124	83.8	24	16.2	12	50.0	2	8.3	10 41.7
Part II	119	81	68.1	38	31.9	20	52.6	6	15.8	12 31.6

cent, were found to contain appreciable amounts of natural amboceptor. A specimen was considered to have an appreciable amount if the largest quantity of inactivated serum used in the complement-fixation test (0.04 c.c.) caused complete or nearly complete hemolysis of 5 per cent cells in fifteen minutes in the water-bath at 37° C. in the presence of two units of complement. The percentage of serums found to contain natural amboceptor in this series is from about one-fifth to one-half as high as that reported by other workers. This is probably due to the use of the shorter incubation period (fifteen minutes instead of sixty minutes at 37° C.) and to the use of only two units of complement (0.005 c.c. to 0.007 c.c. of guinea pig serum) instead of the fixed quantity 0.1 c.c. of a 1:10 dilution. It is also probable that the degree of hemolysis considered to represent appreciable amounts of natural amboceptor has varied with the different workers.

Of all the serums under consideration, 359 were used in the complement-fixation test before and after absorbing the natural amboceptor. Of these,

only 148 reacted at all in the tests read immediately after the hemolysis of controls so that our results (a summary of which is given in the accompanying table) are based upon these 148. Of these 148 reacting serums, 124, or 83.8 per cent, gave exactly the same results before and after absorption of the natural amboceptor. Twenty-four, or 16.2 per cent, gave greater fixation or fixation only after absorption. Of these 24 serums showing an increased degree of fixation after absorption, 12, or 50 per cent, showed a change from no fixation to slight fixation, 2, or 8.3 per cent, showed a change from partial to complete fixation and 10, or 41.7 per cent, showed a slightly increased degree of fixation. But taking 359, the total number of specimens tested before and after absorption (this includes the 212 giving no fixation as well as the 148 giving some fixation), the percentage giving greater fixation after absorption would be 6.6. From the fact that only 12 per cent of all routine serums examined were found to contain appreciable amounts of natural amboceptor, it could be estimated that less than 0.8 per cent would show any difference in reaction because of the removal of natural amboceptor.

When readings of complement-fixation tests were made after they had stood overnight in the cold room, the effect of the removal of natural amboceptor upon the reactions was very much greater than when readings were made immediately after hemolysis of controls. A summary of the results obtained with 119 reacting serums is given in this table. Thirty-eight serums, or 31.9 per cent, gave stronger fixation after absorption than before. Of these 38, 20, or 52.6 per cent, showed a change from no fixation to slight fixation, 6, or 15.8 per cent, showed a change from partial to complete fixation and 12, or 31.6 per cent, showed a slightly increased degree of fixation.

To summarize, our results have agreed with those reported by Olmstead and Kolmer in showing the effect of natural amboceptor to be less apparent if complement-fixation tests are read immediately after the secondary incubation than if read after standing overnight. The natural amboceptor has not been found to affect the complement-fixation reactions to any appreciable degree when readings are made immediately after the hemolysis of serum and antigen controls—so that when this method is followed, the absorption of natural amboceptor does not appear to be essential to the accuracy of the test.

BIBLIOGRAPHY[*]

Bauer, J.: Zur Methodik des serologischen Luesnachweises. Deutsch. med. Wchnschr., 1908, xxxiv, 698.
Hecht, Hugo: Eine Vereinfachung der Komplementbindungsreaktion bei Syphilis, Wien. klin. Wchnschr, 1909, xxii, 338.
Gradwohl, R. B. H.: The Hecht-Weinberg Reaction as a Control over the Wassermann Reaction, Jour. Am. Med. Assn., 1914, lxiii, 240.
Gradwohl, R. B. H.: The Hecht-Weinberg-Gradwohl Test in the Diagnosis of Syphilis, Jour. Am. Med. Assn., 1917, lxviii, 514.
Kaliski, D.: Specificity of the Noguchi Modification of the Wassermann Reaction, Arch. Int. Med., 1910, vi, 205.
Seelman, J. J.: Simple Method of Measuring Antisheep Amboceptor Content of Human Serum and Correcting for it in Wassermann Tests, Jour. Lab. and Clin. Med., 1917-18, iii, 626.

[*]No references to this brief bibliography are given in the text, as a discussion of the work of individual investigators would lengthen the paper unduly.

Stiner, O.: Ueber die Modifikationen der Wassermann'schen Reaktion nach Mintz und Rossi, Ztschr. f. Immunitätsf. u. exper. Therap., xviii, 378.

Rossi, O.: Ueber die Methodik der Wassermann'schen Syphilisreaktion. Ein Verfahren zwecks Absorption der im Menschenserum normalerweise enthaltenen Amboceptoren gegen rote Hammelblutkörperchen. Ztschr. f. Immunitätsf. Orig., 1911, v, 321.

Kahn, R. L.: What the Michigan Department of Health is Doing to Insure Correct Wassermann Results, Public Health, December, 1920, 486.

Ottenberg, R., and Frazier, B.: The Technic of the Wassermann Reaction with Reference to Thomas and Ivy's Method of Complement Dosage and to the Management of Natural Antisheep Amboceptor, Jour. Infect. Dis., 1915, xvi, 119.

Kolmer, J. A., and Rule, A.: The Influence of Natural Antisheep Hemolysin in Human Serum upon the Wassermann Reaction, Am. Jour. Syph., 1920, iv, 135.

Van Saun, A. I.: The Effect of the Natural Antisheep Hemolysin Content of Human Serum on Complement-Fixation Tests, JOUR. LAB. AND CLIN. MED., 1917-18, iii, 59.

Olmstead, M. P.: The Value of Absorption Methods in the Wassermann Test, Med. Rec., 1914, lxxxv, 341.

THE TREATMENT OF RHEUMATIC FEVER WITH THE ETHYL ESTER OF PHENYLCINCHONINIC ACID*

By C. Philip Miller, M.D., and Ralph H. Boots, M.D., New York City

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IN A previous communication[1] we have reported a study of 20 patients suffering from rheumatic fever treated with neocinchophen—the ethyl ester of *para*-methyl phenylcinchoninic acid. This drug was found to exert an antiarthritic and antipyretic action in rheumatic fever comparable to, though slightly less effective than, that of the salicylates. It also produced symptoms of drug intoxication similar to those of salicylism: tinnitus aurium, vertigo, nausea, vomiting, abdominal distress, and signs of renal irritation. These symptoms of toxicity were in general less marked than in salicylate medication. However, it was suggested that as much caution should be exercised in the administration of large doses of neocinchophen as with the administration of sodium salicylate or of acetyl-salicylic acid.

During this clinical investigation of neocinchophen a parallel and more or less comparative study was made of the action of another phenylcinchoninic acid derivative—the ethyl ester of phenylcinchoninic acid.†

These two drugs, neocinchophen and the ethyl ester of phenylcinchoninic acid, seem to be the most efficacious of a large group of compounds which have been prepared in an effort to secure a derivative as potent therapeutically as phenylcinchoninic acid (atophan, cinchophen), but without its

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†This is marketed by the firm of Schering and Glatz, Inc., under the trade-name of Novatophan Improved. Its formula and that of neocinchophen are

COOC₂H

C₆H₅
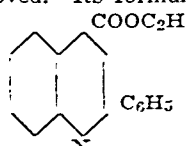
Ethyl ester of phenylcinchoninic acid: Novatophan Improved.

CH₂   COOC₂H₅

C₆H₅
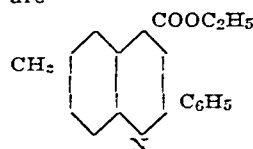
Ethyl ester of *para*-methyl phenylcinchoninic acid:
Neocinchophen
Novatophan
Tolysin

irritating properties. The acid itself exhibits three striking pharmacologic actions:

I. It increases uric acid elimination. This property gave it its first clinical value, and caused it to be quickly adopted as a specific in gout.

II. It relieves the symptoms of rheumatic fever in a manner similar to the salicylates.

III. It restrains the inflammatory reaction to local chemical irritation—its so-called antiphlogistic action—as shown by Starkenstein[2] and by Dohrn[3] who found that rabbits receiving phenylcinchoninic acid by mouth, subcutaneously, or intravenously failed to develop the characteristic conjunctivitis when mustard oil was applied to the eyes. Hanzlik and Tainter,[4] on the other hand, claim that this antiphlogistic action is not demonstrable when other methods of producing local irritation are used.

Many of the derivatives of phenylcinchoninic acid differ therapeutically from the acid itself out of all proportion to their differences in chemical composition. Klemperer[5] has shown that their antigout, antirheumatic, and antiphlogistic properties do not run hand in hand. In fact, some of the compounds retain none of these qualities. According to Dohrn the ethyl ester has no antiphlogistic property in the sense of preventing the mustard oil conjunctivitis in rabbits. Very little information is available concerning its action in rheumatic fever.

Method.—Eight patients ill with rheumatic fever were treated with the ethyl ester of phenylcinchoninic acid. A summary of the clinical data is given in Table I. The method of administration was the same as that described for neocinchophen, i.e., 1 gm. of the drug each hour until the appearance of some evidence of therapeutic effect or of drug intoxication, at which time the drug was discontinued for that day. On succeeding days the patient was kept well under the influence of the drug and as free as possible from toxic symptoms. In other words, we endeavored to maintain the maximum subtoxic dosage. This amount varied considerably and had to be determined for each patient, but in general corresponded to the dosage which we found necessary for neocinchophen. The period of administration varied from four to forty-one days.

Six of the 8 patients treated had severe polyarthritis and one mild polyarthritis at the time of administration. In these 7 patients the arthritis disappeared in 24 to 72 hours after the beginning of medication. In four no relapse occurred. In the three other patients the symptoms were only temporarily alleviated and reappeared when the drug was discontinued. Coincident with the relief of the arthritis there occurred a disappearance of fever and rapid heart rate. As with neocinchophen, there frequently was noted a bradycardia during the first week of administration.

One patient (Case No. V), though relieved of his arthritis by previous treatment with neocinchophen and with aspirin, showed definite signs of persistent infection: low-grade fever, rapid pulse, leucocytosis, and horizontal weight curve. Under the ethyl ester of phenylcinchoninic acid he gradually improved.

TABLE I.

RESULTS OF TREATMENT

CASE HOSP. NO.	SEX AGE WEIGHT	PREVIOUS ATTACKS. AGE 1ST. ATTACK.	DAY OF DISEASE TREATMENT BEGAN	DRUG	AMOUNT OF DRUG 1ST 24 HRS.	AMOUNT OF DRUG TOTAL	THERAPEUTIC EFFECT	TOXIC EFFECT CLINICAL SYMPTOMS	TOXIC EFFECT URINE
							GROUP I: NO RELAPSE AFTER DISCONTINUING DRUG		
I 4718	Male 14 yrs. 51 kgs.	None	7th	Nov. imp.	12 gms. (0.24)*	150 gms. 30 days	Arthritis, fever, and tachycardia disappeared within 2 days. Brady-cardia for 1st 4 days	Slight nausea	Negative before. Tr. albumin and occasional cast throughout administration
II 4658	Male 14 yrs. 49 kgs.	One 5 yrs.	11th	"	11 gms. (0.22)*	140 gms. 31 days	Arthritis disappeared within 24 hrs. Sharp fall of temp. from 105° to normal on 1st day followed by 6 wks. of low grade fever. Pulse subnormal 1st wk., then gradually developing tachycardia. Evidence of severe cardiac involvement	None	Slight increase in albumin and appearance of occasional cast and many renal epith. cells during administration. Negative after discontinuance
III 4733	Male 18 yrs. 44 kgs.	Nine 9 yrs.	6th	"	9 gms. (0.20)*	19 gms. 4 days	Arthritis, fever, and tachycardia disappeared within 24 hrs.	None	No increase in albumin or casts
IV 4724	Male 32 yrs. 48 kgs.	One 22 yrs.	119th	"	8 gms. (0.16)*	112 gms. 26 days	Mild arthritis and fever disappeared within 24 hrs. Pulse slightly rapid for wks.	Nausea and vomiting end of 1st day	Tr. albumin before; no increase. No casts before. Occasional hyaline cast during administration. Many renal epith. cells and few r.b.c. 1st 2 days
V 4513	Male 21 yrs. 58 kgs.	None	29th	Neo.	13 gms. (0.11)*	114 gms. 14 days	Severe arthritis disappeared within 2 days; mild symptoms for 8 more days. Temp. and pulse normal within 3 days. Severe relapse 2 days after drug stopped; treated with aspirin	Slight nausea 2nd day	Tr. albumin before; no increase. Many renal epith. cells, occasional r.b.c. and hyaline casts 1st few days
			45th	Asp.	10 gms. (0.16)*	177 gms. 44 days	Fever and tachycardia disappeared within 24 hrs.; arthritis within 3 days. Mild relapse after aspirin was discontinued; treated with the ethyl ester of phenylcinchoninic acid	Anorexia	Tr. albumin, few casts and r.b.c. 1st 2 days of administration. Disappeared on 3rd day

*No. of grams per kilo body weight.
†Nov. imp. = Ethyl ester of phenylcinchoninic acid.
Neo. = Neocinchophen.
Asp. = Acetyl. salicylic acid (aspirin).

TABLE I—CONT'D

RESULTS OF TREATMENT

CASE HOSP. NO.	SEX AGE WEIGHT	PREVIOUS ATTACKS, AGE 1ST ATTACK	DAY OF DISEASE TREATMENT BEGAN	DRUG	AMOUNT OF DRUG 1ST 24 HRS.	TOTAL	THERAPEUTIC EFFECT	TOXIC EFFECT CLINICAL SYMPTOMS	URINE
VI 4623	Male 13 yrs. 39 kgs.	Six 6 yrs.	98th	Nov. imp.	12 gms. (0.20)*	137 gms. 21 days	Low grade fever and leucocytosis persisted; tachycardia gradually diminished. No joint symptoms	None	No albumin. Occasional hyaline casts 1st day only
GROUP II: RELAPSE AFTER DISCONTINUING DRUG									
			27th	Nov. imp.	13 gms. (0.33)*	125 gms. 25 days	Fever disappeared within 12 hrs. Arthritis and tachycardia within 2 days. Severe relapse immediately after discontinuance of drug	Slight nausea and abdominal distress end of 1st day	Ft. tr. albumin before. No increase. No casts
			61st	Asp.	6 gms. (0.15)*	40 gms. 17 days	Severe arthritis disappeared within 24 hrs. Mild arthritis persisted for 6 days. Fever and tachycardia disappeared within 4 hrs.	Tinnitus, nausea, vomiting, deafness, and vertigo 1st day	Slight increase in albumin, many renal epith. cells and occasional r.b.c. 1st 48 hrs.
VII 4622	Female 44 yrs. 63 kgs.		218th	Nov. imp.	17 gms. (0.27)*	168 gms. 16 days	Fever disappeared within 12 hrs. Tachycardia and severe arthritis within 48 hrs. Mild stiffness persisted throughout administration. Relapse began 9 days after dis continuance of drug	None	Ft. tr. albumin before; no increase. Many renal epith. cells during administration. Occasional cast during and following administration
			240th	Asp.	8 gms. (0.12)*	52 gms. 24 days	Fever and tachycardia disappeared within 8 hrs. Arthritis entirely disappeared within 48 hrs.	Slight tinnitus, vertigo and nausea 1st 4 days	Ft. tr. albumin before; no increase. Occasional casts before; increased 1st 5 days. Many renal epith. cells and occasional r.b.c. 1st 2 days
VIII 4367	Male 19 yrs. 61 kgs.	Three 14 yrs.	6th	Nov. imp.	10 gms. (0.16)*	260 gms. 41 days	Fever and tachycardia disappeared within 24 hrs. Arthritis within 2 days. Arthritis reappeared on 10th day and persisted. Severe relapse occurred while dosage was being decreased	Slight tinnitus and vertigo 1st 2 days	Tr. albumin before; not increased; disappeared during administration. Occasional casts 2nd day only
			48th	Asp.	7 gms. (0.11)*	190 gms. 40 days	Arthritis disappeared within 24 hrs. Fever and tachycardia within 48 hrs.	Tinnitus, deafness, vertigo nausea and vomiting 1st 2 days	Negative before. Tr. albumin 1st 2 days only. Occasional casts throughout administration

This drug also gave rise to toxic symptoms similar in character to those of salicylism but milder in degree. While no definite general conclusions can be drawn from this small series of cases the possibility is suggested that the ethyl ester of phenylcinchoninic acid is less toxic than neocinchophen.

One patient, Case No. V (mentioned above), and the three who suffered relapses after medication was withdrawn and were subsequently treated with aspirin, furnished the opportunity to compare as to therapeutic efficiency and toxicity the ethyl ester of phenylcinchoninic acid and aspirin— one of the established salicylate antirheumatics. The comparison corresponds closely to that of neocinchophen and the salicylates.[1] In brief, the latter seem to be somewhat more effective in relieving the symptoms of rheumatic fever and at the same time more toxic.

CONCLUSION

The ethyl ester of phenylcinchoninic acid corresponds closely to neocinchophen as an antisymptomatic remedy for rheumatic fever.

REFERENCES

[1]Boots, Ralph H., and Miller, C. Philip: Jour. Am. Med. Assn., 1924, lxxxii, 1928.
[2]Starkenstein, E., and Wiechowski: Prag. med. Wchnschr., 1913, xxxviii, 36.
[3]Dohrn, Max: Die Therapie der Gegenwart, 1913, liv, 196.
[4]Hanzlik P. J., and Tainter, M. L.: JOUR. LAB. AND CLIN. MED., 1923, ix, 166.
[5]Klemperer, G.: Die Therapie der Gegenwart, 1913, liv, 257.

ORGANIC, PROTEIN AND COLLOID SILVER COMPOUNDS

III. DOES THE "COLLOIDAL SILVER" BECOME AVAILABLE AS ANTISEPTIC?*

BY TORALD SOLLMANN AND J. D. PILCHER, CLEVELAND, OHIO

INTRODUCTION

AS has been shown in the previous papers of this series,[1] the growth of yeast cells is restrained by silver ions. The immediate inhibiting action of the various "colloidal" and "protein" silver compounds is due to the silver ions which are present in some degree in all the compounds of this class. When these are deducted, there remains a very considerable amount of silver which, under the conditions of the experiment, takes no part in the antiseptic effect and which may be designated as "inactive silver" (Table I). It consists mainly of mixtures of metallic silver and of silver oxides, perhaps also of obscure silver protein compounds, all in colloidal form.

TABLE I

PERCENTAGES OF ACTIVE AND INACTIVE SILVER IN SOME OF THE MORE IMPORTANT COMPOUNDS

COMPOUND	TOTAL SILVER CONTENT	ACTIVE SILVER	INACTIVE SILVER
	%	%	%
Silver Nitrate	63.5	63.5	0
Albargin	15.0	13.3	1.7
Protargol	8.3	7.5	0.8
Collargol	78.	1.5	76.5
Argyrol	22.5	0.4	22.1
Silvol	20.0	0.2	19.8

Assuming it to be established that this colloidal silver plays no part in the immediate antiseptic action, there remains the possibility that it might become gradually converted into active ionic silver, either by spontaneous progressive decomposition of the colloids, or to replace such ionic silver as is bound or precipitated in the course of the antiseptic action. This theory, that the colloidal silver constitutes a "reserve" of potential silver ions, which prolongs the antiseptic efficiency, prevails quite widely, i.e., while it is universally conceded that the colloidal silver compounds are much weaker than silver nitrate, when the contact is short, it is often asserted that they become (relatively) much more active if the contact is prolonged. It must be pointed out, however, that this assumption is essentially speculative; no objective evidence that would stand reasonable criticism is available.

The present investigation was intended to answer this question; i.e., whether the colloidal silver constitutes "reserve-silver," that might become available under suitable conditions; or whether it remains inert, serving merely as a colloidal demulcent.

The problem was approached from several angles, as will be seen.

I. THE INFLUENCE OF TIME OF CONTACT ON THE RELATIVE EFFICIENCY OF SILVER COMPOUNDS

If the colloidal silver is capable of gradual transformation into antiseptically active silver, its importance would increase with the duration of the contact of the yeast with the silver compound; i.e., the efficiency ratio: $\frac{\text{colloidal compound}}{\text{silver nitrate}}$ would increase with the duration of the experiment.

To test this matter, the method previously described was modified so that six hours, instead of one hour, would be required to form 10 cm. of gas. This is easily accomplished by reducing the percentage of yeast; for it was found that the time required to produce a given quantity of gas varies approximately as the reciprocal of the concentration of yeast, the sugar percentage remaining constant; i.e., if it is wished to slow the gas formation to one-half or one-fourth, it suffices to reduce the percentage of yeast to one-half or one-fourth. The agreement is sufficiently close. Another method for slowing the gas formation consists in reducing the temperature. In our principal series, the two measures were combined; incubating 2 per cent yeast with 10 per cent cane sugar at 21° C. (instead of the 4 per cent of

yeast at 38° C., used in the standard one-hour method). This yields 10 cm. of gas in six hours. Hourly observations were made. Four different concentrations of compounds with widely different proportions of colloidal silver (see Table I) were used; namely, silver nitrate, protargol, argyrol, and collargol.

The point of the experiments, then, was this: If the colloidal silver becomes gradually activated, the ratio of gas formed in the colloidal compounds, as compared with that formed in silver nitrate, should *decrease* from the earlier hours to the later hours. If, on the other hand, the two run parallel, or if the ratio increases with the time, then the colloidal silver has remained inert.
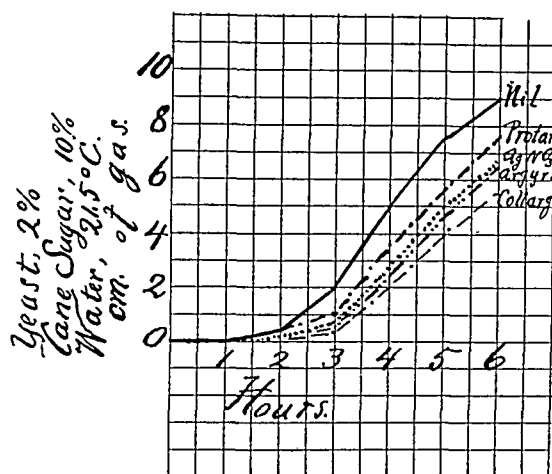
Curve 1.—Rate of gas formation with different silver compounds.
Note that the curves are essentially parallel; if the reserve silver had become active, the curve would be relatively high in the early periods, and relatively low in the later periods.
——————— Unpoisoned solution.
. . . . Silver-nitrate, 0.1 mg.
-.-.-.-. Protargol 1.5 mg.
-..-..- Argyrol 10 mg.
--.--.--. Collargol 5 mg. (all per 10 c.c. of mixture).

The outcome of all the series of experiments concurs in showing that the ratio of gas formation does *not* decrease with time; i.e., they show that the *colloidal silver remains inert;* and this whether the concentration of active ions is relatively low or relatively high; whether the experiments are made

TABLE II

QUANTITY OF GAS LIBERATED IN WATERY MIXTURE OF YEAST 2 PER CENT, CANE SUGAR 10 PER CENT, INCUBATED AT 21.5°C:

HOURS	CM. OF GAS			RATIO OF GAS, COMPARED WITH AGNO$_3$=1		
	2	4	6	2	4	6
Products and dosage (mg. per 10 c.c.):						
AgNO$_3$ 0.1 mg.	*	2.7	6.5	1.0	1.0	1.0
Protargol 1.5 mg.	0.3	3.5	7.5	*	1.3	1.2
Argyrol 10. mg.	*	2.5	6.5	1.0	0.9	1.0
Collargol 5 mg.	0	2.0	5.5	—	0.74	0.85
Control unpoisoned	0.3	5.0	10.0	*	1.8	1.5

*Present, but too small for measurement.

TABLE III.

As in Table II, but Normal Saline in Place of Water.

HOURS	CM. OF GAS			RATIO OF GAS, COMPARED WITH $AgNO_3=1$		
	2	4	6	2	4	6
Product and dosage (mg. per 10 c.c.):						
$AgNO_3$ 2 mg.	1.5	4.5	8.0	1.0	1.0	1.0
Protargol 10 mg.	0.4	5.5	10.0	0.27	1.2	1.3
Argyrol 5 mg.	0.8	4.5	8.5	0.53	1.0	1.1
Collargol 10 mg.	0.3	2.7	6.0	0.2	0.6	0.67

in water or in normal saline; with 2 per cent of yeast at 21° C.; or with ½ per cent at 38° C.: under none of these conditions does the silver become available.

For illustration, we may quote the figures of curves of a typical experiment in water (Table II and Curve I) and in normal saline (Table III).

The figures are quoted only for 2 hour intervals for the sake of simplification.

It must therefore be concluded that the nonionic silver of colloidal silver compounds does not become converted into ionic silver during six hours of contact with yeast mixtures; it is therefore antiseptically inert, and cannot be considered as "reserve-silver."

II. THE QUANTITY OF SILVER AVAILABLE AFTER COMPLETION OF THE STANDARD EXPERIMENT

It may be assumed that the active, i.e., ionic silver produces its effects by combining with the protoplasm of the yeast, and that it is thereby rendered inactive. This is confirmed by the observation that a given quantity of silver can paralyze only a limited quantity of yeast (E. Zerner and R. Hamburger).[2]

The "active" silver can, therefore, be promptly and more or less completely removed by digesting with yeast. "Reserve-silver" would not be affected by this, except in proportion as it became converted into active silver.

If, therefore, a just-inhibitory dose of a silver-compound is added to a yeast mixture and digested for an hour, the active silver will be more or less completely bound, but the greater part of any "reserve-silver" would be still available. If, then, fresh yeast is added and again digested, the gas formation in the second digestion would be restrained distinctly more by preparations containing utilizable "reserve-silver," than by those in which no silver reserve is mobilized; and if a preparation contains utilizable reserve-silver, its antiseptic efficiency in the second fermentation should be greater than with silver nitrate

The experiments were conducted in the usual manner, by digesting at 38° C., a 4 per cent suspension of yeast in 10 per cent cane-sugar solution, containing the just-inhibitory concentrations of the silver compounds, and observing the gas formation at the end of an hour. The tubes were then emptied, and the mixture shaken, or rubbed with 1 or 4 per cent of fresh

yeast. They were then re-mounted, digested for a second hour, and again observed.

When 4 per cent of yeast was added in the second digestion, the gas formation was so active that practically all the tubes were completely filled with gas; so that no quantitative conclusions could be drawn.

The average figures for the other experiments are shown in Table IV; the individual data agreeing well with the averages.

TABLE IV

GAS FORMATION IN YEAST MIXTURES ON 1ST. AND 2ND. DIGESTION

	FIRST DIGESTION 4% YEAST (A)	SECOND DIGESTION	
		WITHOUT FURTHER YEAST (B)	WITH 1% OF FRESH YEAST (C)
Silver nitrate 0.45 mg. per 10 c.c.	0.6 cm.	2.0 cm.	4.5 cm.
Protargol, 4.0 mg. per 10 c.c.	0	1.6.	3.5
Argyrol 35 mg. per 10 c.c.	0	0.5	3.75

Column C shows somewhat less restraint by silver nitrate than by the other compounds, in the second digestion; but the silver nitrate was also somewhat less efficient in the first digestion and this accounts fully for the small difference in the second digestion.

This line of experimentation therefore leads to the same result as the first line; i.e., that the "colloidal silver" in the silver compounds is useless as an antiseptic.

CONCLUSIONS

The "colloidal silver" of the "colloidal and protein-silver compounds" does not exert any antiseptic action even after six hours of contact; the entire antiseptic efficiency resides in the silver ions that are present or become immediately available. Whatever value the colloidal silver has must depend upon demulcent qualities, which would serve to diminish the irritative effects of the antiseptic silver ions. It is therefore very doubtful whether the colloidal part of the silver compounds plays any greater part in their therapeutic action than would other indifferent colloids such as the proteins that occur in the same compounds.

BIBLIOGRAPHY

[1]Pilcher, J. D., and Sollmann, T.:   JOUR. LAB. AND CLIN. MED., 1923, viii, 301; erratum, ibid., p. 462; 1924, ix, 256.
[2]Zerner, E., and Hamburger, R.:   Biochem. Ztschr., 1921, cxxii, 315.

A QUANTITATIVE COMPARISON OF THE TOXICITY OF THE ALKAMINE ESTERS OF AROMATIC ACIDS USED AS LOCAL ANESTHETICS*

BY CHARLES W. HOOPER, A.B., M.D., AND ELSA BECKER, A.B., NEW YORK CITY

ACCURATELY controlled animal experiments showing the relative toxicity of the various drugs used as local anesthetics may have a direct clinical application in supplying unbiased data by which the relative safety of the drugs may be estimated.

The toxicity of the local anesthetics has been so widely discussed recently that we will confine ourselves to the results we have obtained by a method which from an experimental viewpoint may be considered quantitative.

Professors Hatcher and Eggleston, 1919, were the first to carry out comprehensive experimental work to determine the relative toxicity of all of the drugs used as local anesthetics. They realized that the toxic action of the local anesthetics is exerted only after the entrance of the drugs into the blood stream and therefore that the most accurate method of determining the relative toxicity of the local anesthetics is to determine the maximal toxicity of each after intravenous administration. They determined the maximal toxicity of each by rapid intravenous injections of water solutions of the drugs into cats. The concentration of the solutions varied from one to twenty per cent so that the dose could be injected from a syringe in from five to fifteen seconds.

In view of the fact that the ratio of toxicity of each of the local anesthetics varies greatly in different species of animals when injected subcutaneously, we decided to determine the maximal toxicity by intravenous injection in some species other than the cat and also to see whether we could develop a method which from an experimental viewpoint might be considered quantitative. For this purpose we adopted a test based upon the official method used by the Hygienic Laboratory, United States Public Health Service, and the manufacturers to determine the toxicity of arsphenamine and related products before they are released for distribution. The Government selected the rat for this test because the results obtained seemed to be more constant than with other experimental animals. Furthermore, on account of their small size, a large number of rats can be kept conveniently under uniform conditions of diet, weight, etc., and as a result more animals can be used for an individual test.

We have applied a modification of the official technic for the determination of the maximal toxicity of the alkamine esters of the aromatic acids

used as local anesthetics and have found that the results obtained are very accurate and correspond very closely to those. obtained with the cat by Professors Hatcher and Eggleston.
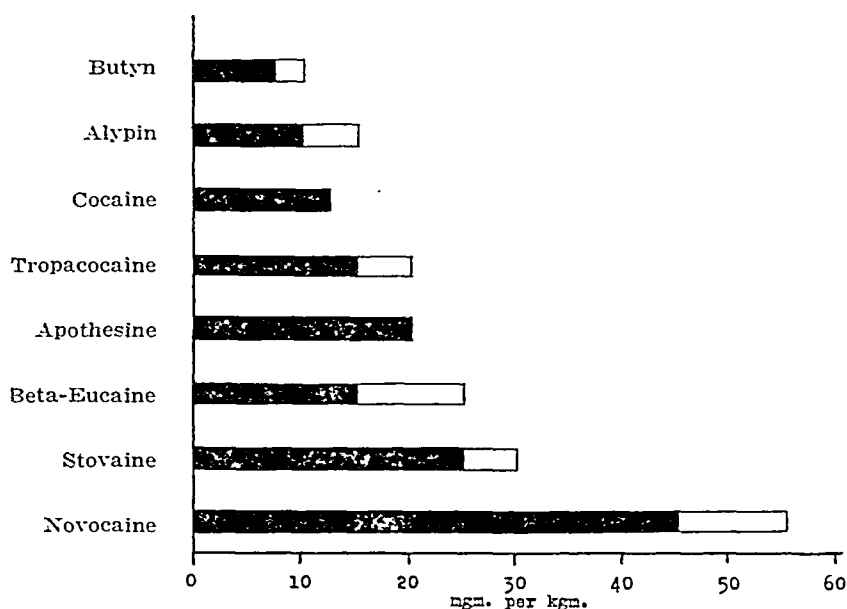

Chart 1.—A Quantitative Comparison of the Toxicity of the Alkamine Esters of Aromatic Acids used as Local Anesthetics. Maximal Tolerated and Minimal Lethal Dose in Milligrams per Kilo Body Weight.

TECHNIC OF THE TEST

Healthy nonpregnant albino rats weighing between 100 and 150 grams were employed. The rats were obtained from one source and were kept on a constant diet for at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs when they were weighed and the doses administered per kilo body weight. Water was supplied during the fast period. A 2 per cent solution

TABLE I

COMPARATIVE VARIATION IN THE TOXICITY RESULTS OBTAINED IN THE ALBINO RAT AND CAT AFTER INTRAVENOUS INJECTION OF THE ALKAMINE ESTERS OF THE AROMATIC ACIDS USED AS LOCAL ANESTHETICS

	ALBINO RATS		CATS	
	MAXIMAL TOLER-ATED AND MINIMAL LETHAL DOSES	STRENGTH OF SOLUTION	FATAL DOSE	STRENGTH OF SOLUTION
	mg. per kg.	per ct.	mg. per kg.	per ct.
BUTYN	7.5-10	2	15*	1
ALYPIN	10 -15	2	10**	5-10
COCAINE	12.5	2	15**	5-10
TROPACOCAINE	15 -20	2	18-22**	10
APOTHESINE	20	2	20**	5-10
BETA-EUCAINE	15 -25	2	10-12.5**	5
STOVAINE	25 -30	2	25-30**	10
NOVOCAINE	45 -55	2	40-45**	5-20

*Fatal dose obtained by Prof. Robert A. Hatcher. See Bonar, M. L., and Sollmann, Torald: Jour. Pharmacol. and Exper. Therap., 1922, xviii, 475.

**Fatal doses obtained by Prof. Cary Eggleston and Prof. Robert A. Hatcher: Jour. Pharmacol. and Exper. Therap., 1919, xiii, 444.

TABLE II

A Quantitative Comparison of the Toxicity of the Alkamine Esters of the Aromatic Acids Used as Local Anesthetics. Rats Injected Intravenously. Percentage of Rats that Died. (The Numbers within Parentheses Represent the Number of Rats Used in the Series)

	DOSE EXPRESSED IN MILLIGRAMS PER KILO BODY WEIGHT													
	5	7.5	10	12.5	15	17.5	20	25	30	40	45	50	55	60
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
BUTYN	0(6)	20(5)	70(10)		100(5)									
ALYPIN	0(5)		14(7)		71(7)		100(7)							
COCAINE			0(5)	60(5)	80(5)		80(10)	100(3)						
TROPACOCAINE			0(5)		20(5)		100(5)							
APOTHESINE						0(5)	100(5)		100(5)					
BETA-EUCAINE			0(5)		0(5)		80(5)	100(5)						
STOVAINE					20(5)		40(5)	100(5)	100(9)					
NOVOCAINE							0(5)	20(5)	0(5)	0(14)	17(30)	25(40)	57(23)	75(24)

of the drug in distilled water was injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 c.c. At least five rats were injected at each dose. The animals were kept under observation for twenty-four hours, during which time they were fed the routine diet. The minimal dose at which any of a series of animals died is considered the maximal tolerated dose. The dose at which the majority of a series died is considered the minimal lethal dose.

The abscissae represent the number of milligrams of the drugs injected per kilo body weight. The shaded portions of the ordinates show the maximal tolerated doses, the unshaded portions the minimal lethal doses. The chart shows that butyn is the most toxic of the alkamine esters of the aromatic acids used as local anesthetics. The maximal tolerated dose is 7.5 milligrams and the minimal lethal dose 10 milligrams. The maximal tolerated dose of cocaine as well as the minimal lethal dose is 12.5 milligrams. The maximal tolerated dose of apothesine as well as the minimal lethal dose is 20 milligrams. The maximal tolerated dose of novocaine is 45 milligrams and the minimal lethal dose is 55 milligrams.

In the first column are given the local anesthetics employed; in the second column the maximal tolerated and minimal lethal doses for the albino rat in milligrams per kilo body weight; in the third column the strength of the solution injected into the saphenous vein of the rat; in the fourth column the fatal dose for the cat obtained by Professors Hatcher and Eggleston by their rapid intravenous injection method and in the fifth column the strength of the solution injected.

It will be noted that the results obtained by the two methods are quite comparable and that there is very little variation in the maximal toxicity for the two species when the drugs are injected intravenously.

It will be noted that the toxicity results obtained by injecting the local anesthetics intravenously into albino rats by the above described method are clean cut. The difference in the individual susceptibility of the animals is slight. The variation between the maximal tolerated dose and the minimal lethal dose does not exceed ten milligrams in any of the experiments. The results indicate that the method from an experimental viewpoint may be considered quantitative.

LABORATORY METHODS

AN ADJUSTABLE DOUBLE-SLIT LAMP FOR USE IN MULTIPLE OPTICAL REGISTRATIONS*

By Louis M. Katz, A.M., M.D., and William R. Baker, Cleveland, Ohio

INTRODUCTION

THE successful employment of optical manometers and similar types of apparatus involves not only a considerable amount of experience but also requires careful attention to a large number of details before satisfactory photographic records can be obtained. Efforts are therefore being made in this laboratory to simplify the practical aspects of such registration. The adjustable double-slit lamp described in this communication was constructed in order to facilitate the projection of light beams upon mirrors of several optical recorders, in such a way as to avoid parallax in the reflected beams. This lamp has been successfully employed during the past year.

PRINCIPLES OF OPTICAL REGISTRATION

To understand the advantages of the lamp it is necessary to recall the essential principles which underlie optical registration. The clearness of optical records is determined to a large extent by the arrangement of the three essential parts of an optical system, namely, the recording camera, the projecting lamps and the reflecting mirrors on the manometers or segment capsules. A good curve is obtained when the beam of light striking the camera slot is focused and has sufficient luminosity, and when the movements of the reflecting mirror are adequately magnified. The focus of the beam is readily adjusted by shifting the position of the projecting lens. The luminosity of the reflected beam depends (a) on the type of lamp used, (b) on the distance between the mirrors and the camera, (c) on the proximity of the mirrors to the focus of the source of light, (d) on the width of the reflecting mirrors.

It has been the experience in this laboratory that the best magnification is obtained, with manometers and segment capsules of proper vibration frequency, when the distance between the mirror and camera is approximately 120 cm. In order to maintain a proper vibration frequency, relatively small mirrors (2 to 4 mm. in width) must frequently be used. At this distance, and with such small mirrors, the light value of a Nernst lamp is insufficient except for very slowly moving photographic paper. The only source of light

so far available under these conditions has therefore been the D.C. arc lamp, a very useful model of which has been devised by Wiggers.[1]

It is often necessary, both in the clinic and the research laboratory, to record more than one curve at the same time. In general, such optical records are obtained either by using several lamps, one for each manometer or segment capsule, or by using a single lamp constructed to project more than one beam of light. In comparing the time relationship of such simultaneously recorded curves the question of parallax must be taken into account. And probably nowhere is this so important as in the modern exact optical studies of cardiodynamics. The duration of the phases of the heart cycle are so brief and occur in such rapid succession that even a slight uncorrected parallax may lead to a significant error. A little reflection will convince one that the presence or absence of parallax depends entirely on
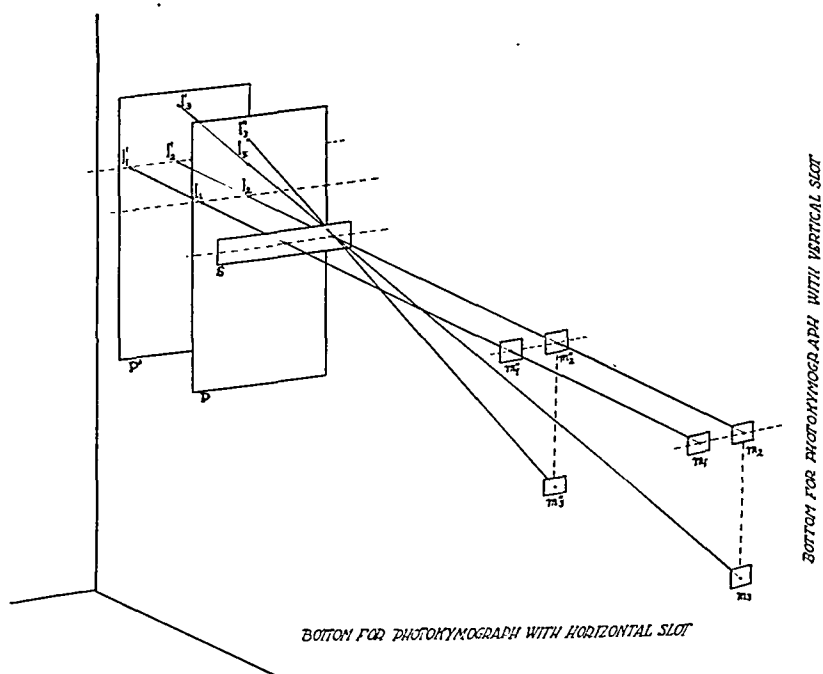

Fig. 1.—A three plane diagram showing how the relation of the mirrors and photographic surfaces to the camera slot modifies the amount of parallax. S represents the camera slot; P and P', two positions of the photographic surface; I_1, I_2 and I_3, the images of beams of light reflected respectively from mirrors m_1, m_2 and m_3 on the photographic surface P; I'_1, I'_2 and I'_3, the images from the same mirrors on photographic surfaces P'3; I''_3, the image of beam from mirror m''_3 on photographic surface P. The images from m''_1 and m''_2 are the same as those from m_1 and m_2. Further description in text.

the arrangement of the reflecting mirrors with regard to the axis of the cylindrical lens of the photokymograph. Parallax is absent when the centers of the reflecting mirrors are in the same plane as the axis of the camera slot (m_1 and m_2, m''_1 and m''_2 in Fig. 1); this is to say the mirrors must be horizontal when the camera slot is horizontal and over one another when the slot is vertical. Parallax is present *only when the above condition is not fulfilled* and its amount depends on (1) the displacement of the mirrors from the plane of the camera slot, (2) the distance between the mirrors and the lens of the photokymograph and (3) the distance between the lens and the photographic surface. The greater the displacement of the mirrors from the plane of the camera lens, that is the greater the distance between m_2 and

m_3 in Fig. 1, the larger will the parallax become because the angle between the two beams will increase. The amount of parallax will also be increased if the mirrors are brought closer to the camera (compare images of m_2 and m_3 with those of m''_2 and m''_3 in Fig. 1) or if the photographic paper is moved away from the lens of the camera (compare I_2 and I_3 with I'_2 and I'_3 in Fig. 1). In the former case this is due to a greater angle between the two beams of light and in the latter to the greater separation of the beams as they travel back.

The significance of inattention to parallax is not always the same but depends on the rapidity with which the photographic surface moves past the slot, for the time value of each linear unit is much less at rapid rates than at the slower ones. The above statements can be summed up in the following formula:

$$p = \frac{f.m}{r.s}$$

in which p is the time value of the parallax; f, the distance from lens to photographic paper; m, the distance between the two mirrors in a plane parallel to the photographic surface and perpendicular to the axis of the camera lens; r, the average distance from the recording mirror to the camera lens; and s, the speed with which the photographic surface moves past the camera slot.

For practical reasons it has not always been possible to obviate parallax and therefore various methods have been introduced for evaluating it. For the most part they consist either in photographing the beams of light when the photographic surface is stationary, preferably through amber glass (Garten[2]) or in simultaneously interrupting the light beams while the records are being obtained.

CRITIQUE OF OTHER LAMPS

How do the various types of optical systems used in the past compare according to these principles? Otto Frank[3] recognized that the recording of simultaneous curves can be simplified by using a single lamp which projects more than one light beam. He accomplished this by providing a Nernst filament lamp with a triple objective. The beams of light from his lamp are focused on the several mirrors either by approximating or separating the mirrors (a procedure not always possible) or by varying the distance between the mirrors and the lamp. In other words, the amount of adjustment with this lamp is rather limited. C. Tigerstedt[4] modified the "Frank" lamp in two essential details for this reason. His lamp has five objectives instead of three, each of which is movable independently of the others. A wider range of adjustment is therefore possible with Tigerstedt's lamp but yet not sufficient for all purposes. Moreover, parallax cannot be avoided when using either of the lamps, a fact that these investigators recognized and corrected by interrupting the several light beams simultaneously.

H. Straub[5] obtained a double beam of light from a single lamp by another principle. He projected two closely placed parallel Nernst filaments through a single objective and claimed that in this way he avoided parallax entirely,

or at least reduced it to a negligible factor. Inasmuch as the two filaments are fixed, Straub's lamp has to be adjusted in the same way as Frank's and therefore has the same disadvantages. In addition to the drawbacks just mentioned all of the Nernst filament lamps lack the luminosity of the arc lamp and moreover they do not permit a variation in the width of the beams.

The arc lamp constructed by Wiggers,[1] which projects a band of light by means of a slit of adjustable width, can also be focused on several mirrors. It allows a good deal of adjustment of the mirrors particularly in the axis of the light band. Parallax is always present, however, because the axis of the light band is at right angles to the axis of the camera slot and therefore does not allow one to place the mirrors in the plane of the camera slot. Wiggers has minimized the amount of parallax to 0.75 mm. when the
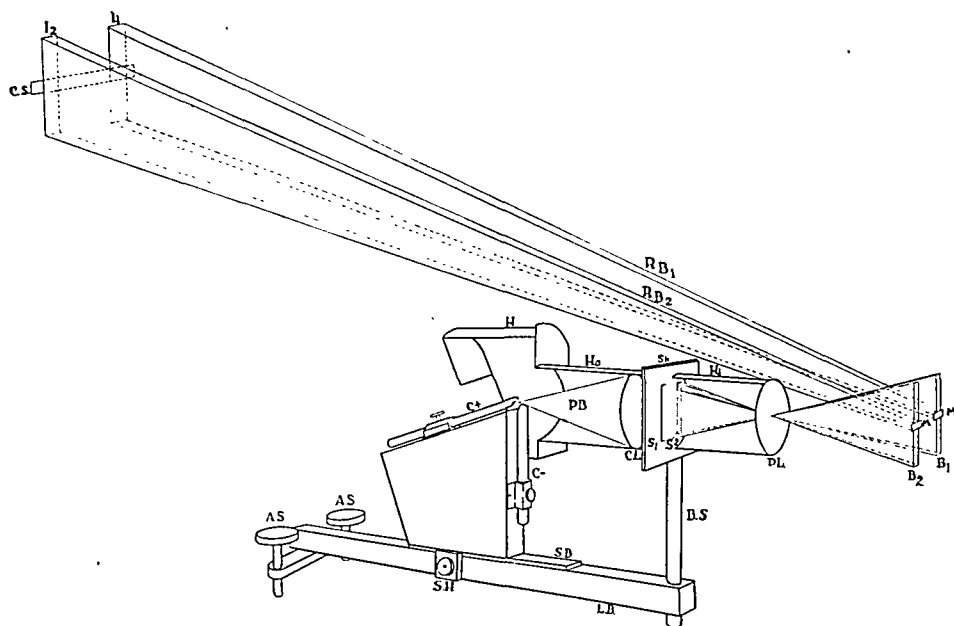

Fig. 2.—A three plane diagram of the double-slit lamp showing the important details of its construction. The side of the hood H and the draw tubes H_0 and H_1 toward the observer have been removed in this diagram in order to show the course of the light beams. Description is given in text.

mirrors are 120 cm. from the camera by placing a narrow hood 16 cm. long in front of the camera slot. The parallax in the system is determined by Garten's method.

THE ADJUSTABLE DOUBLE-SLIT LAMP

The adjustable double-slit lamp which we ·built and which is described in this communication combines the valuable features of the preceding lamps and at the same time obviates many of their difficulties. With it we are able to project two parallel bands of light, to adjust the width of each of the bands *independently* and above all *to vary the distance between them.* The luminosity of this lamp is sufficient even at a distance of 120 cm. and with bromide paper moving past the camera slot at a rate of 135 mm. per second as proved by actual test; an efficiency reached only by Wiggers' arc lamp. There is no difficulty with focusing and there is a large margin for adequate magnification of the curves. Parallax can readily be avoided simply by see-

ing that the two mirrors lie in the plane of the camera lens. In fact we have calculated by the formula given and proved by actual experiment that the amount of parallax produced when the mirrors are separated 1 cm. in a plane at right angles to the camera slot, is less than 0.15 mm. The ability to shift the bands of light independently allows a greater freedom in the placement of the manometers than can be attained with any other lamp heretofore described, a feature of great significance when studying the pressure changes in the various heart chambers. Adjustments of the various manometers (or capsules) can be made without throwing everything out of line; all that is required is to shift the band of light concerned to a new position.

A schematic drawing of the lamp and the course of the light beams is shown in Fig. 2. The principle employed is to bring the light which passes through the two variable slits S_1 and S_2 on the exact optical center of a projecting lens (P.L.) by means of a condensing lens (C.L.) and then to allow the light to spread as two bands B_1 and B_2 to be reflected from two mirrors (M) as rays RB and RB_2. The details of the lamp are as follows: A 4-ampere 110 D.C. Spencer arc lamp (C−, C+) is mounted on a bar (S.B.) which slides on a long steel bar (L.B.) 2.5 cm. in diameter and 53 cm. long. The lamp is fixed in position by tightening a set screw (S.H.). The whole apparatus may be tilted at various angles by the set screws (A.S.). These are 9 cm. in length and are set in a cross bar which is fastened to the back of the long steel bar. Each of the screws is held in position by means of a lock nut to enhance rigidity.

The projection system which consists essentially of a condensing lens (C.L.), a projecting lens (P L) and an adjustable double-slit mechanism (Sh.), is supported in a circular flat steel clasp resting on the top of the upright bar (B.S.). With this arrangement the projection system can be revolved around its long axis so that the bands of light can be projected perpendicular to the camera slot at all times, regardless of whether the slot is vertical or horizontal.

The condensing lens is an ordinary biconvex lens 5 cm. in diameter with a principle focus of 9 cm. It is used to bring the light (P.B.) from the positive carbon (C +) to a focus in the center of the projecting lens (P.L.). In other words the arrangement is such that the light from the two slits comes to a point in the optical center of the projecting lens, regardless of their width or position. Spherical and chromatic aberration are prevented in this way even when inexpensive lenses are employed.

The projecting lens is also biconvex and has the same diameter and focus as the condensing lens. It is mounted in a telescoping draw tube (H_1) in front of the slit mechanism and it can be placed at any distance from 10 to 18 cm. in front of the slits by adjusting the draw tube. This adjustment allows the images I_1 and I_2 to be focused sharply on the camera slot (C.S.).

The distance between the slit mechanism and the condensing lens is fixed at 4 cm., the light from the positive carbon being focused on the projecting lens by shifting the position of the arc lamp on the long bar. The space between the arc lamp and the condensing lens is made light-tight by a tele-

scoping draw tube (H$_o$) which allows the lamp to be placed anywhere from 9 to 35.5 cm. behind the lens.

The principal axis of the entire optical system is parallel to the long bar (L.B.) and 15 cm. above it. In most of our work we have set the system at the following distances: Positive carbon to condensing lens 21.5 cm.; condensing lens to slits 4 cm.; slits to projecting lens 10 cm.; projecting lens to mirrors 10 cm.; mirrors to camera lens 120 cm.; camera lens to photographic paper 1 cm.
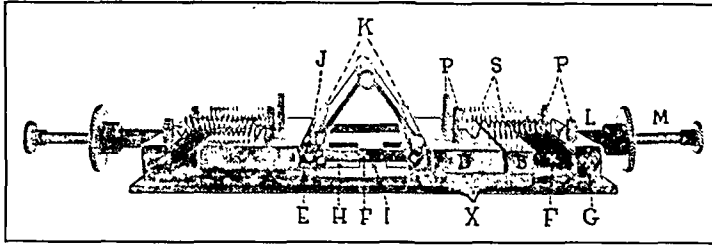

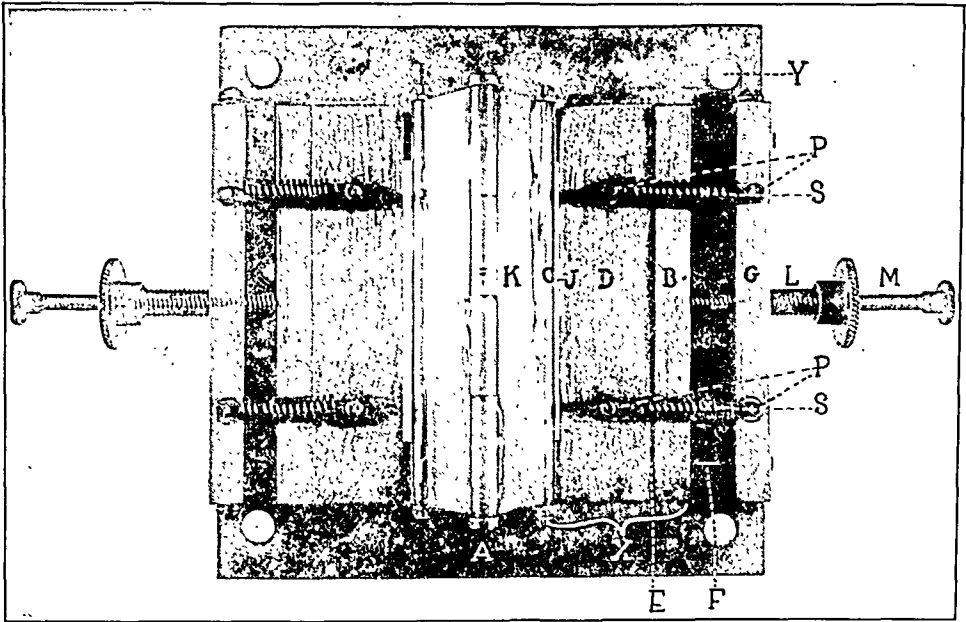Fig. 3.—Side view of the shutter mechanism described in text.

Fig. 4.—Front view of the shutter mechanism described in text.

THE DOUBLE-SLIT SHUTTER MECHANISM

The construction of an adjustable double-slit mechanism constituted a mechanical problem of the greatest difficulty. Figs. 3 and 4 are introduced to make clear the details of its structure as it was finally built. It consists of a brass aperture plate (A) 7.5 cm. square and 1.6 mm. thick, on which are mounted six sliding bars, 3.5 mm. thick and 5.5 cm. long; the two outer bars (B) being 4.8 mm. wide, the two inner ones (C) 2.8 mm and the intermediate bars (D) 12.7 mm. The aperture plate has two oblong openings (H), 12 mm. by 40 mm., which are separated by a rib of metal (I) 4.8 mm. wide. The inner and outer bars on each side are connected at the top and bottom

by two tubes (E) 3.2 mm. in outer diameter and 1.6 mm. bore, the distance between the bars being fixed at 19.2 mm. This constitutes the sliding frame (X). The two tubes of this frame slide on two parallel round rods (F) which are 1.6 mm. in diameter and 7.5 cm. long. The rods are mounted so as to form an angle of exactly 90° with the rib of the aperture plate and are fastened at either end to a bar (G) which is screwed to the aperture plate. The intermediate bar (D) slides on the tubing of the sliding frame. The space between the inner and outer bar forms the slit opening on each side (J), the under surface of the bars adjoining the slit being beveled to form a knife edge. The space between the inner bars of the two sliding frames is bridged by a metal hinge (K) which forms a light-tight partition and prevents light from passing through this space.

The position and width of each slit can be altered *independently* by means of four thumb screws (L and M), two on each side, which work against four tension springs (S). The tension springs are fastened to pins (P) placed in the slide rod supports (G) and the intermediary bar (D). This arrangement allows the springs to exert an outward pull both on the intermediary bar and the sliding frame (X). The outer thumb screw (L) which is hollow is threaded through the slide rod support (G) and presses against the outer side of the outer bar (B). When it is screwed in, it forces the sliding frame and with it the slit towards the mid-line. When it is screwed out the sliding frame is pulled away from the mid-line by the tension springs. The inner thumb screw (M) passes through the hollow outer thumb screw (L), is threaded into the outer bar (B) of the sliding frame and presses against the intermediate bar (D). When it is screwed in, it forces the intermediate bar towards the mid-line narrowing the slit. When it is screwed out the tension springs pull the intermediate bar outward and widen the slit.

The width of each slit (J) can be varied from 0 to 1 mm. The distance between the slits can be varied from 6.4 to 25.4 mm. When the mirrors are placed 10 cm. in front of the projecting lens it means that the mirrors can also be shifted a like amount.

The aperture plate of the shutter mechanism is fastened to a plate on the end of the metal tube which holds the condensing lens. A piece of asbestos 3.5 mm. thick is interposed between these plates in order to prevent the conduction of heat to the shutter mechanism by the metal. The telescope tube holding the projecting lens has a plate at its free end which is also fastened to the aperture plate by four threaded pins at (Y). A space of 2 cm. is, however, left behind the shutter mechanism which allows a free circulation of air and helps to prevent an excessive rise in its temperature. For the sake of clarity these attachments have been omitted in the diagram given in Fig. 2.

REFERENCES

[1]Wiggers: Circulation in Health and Disease, 1923, ed 2, p. 179.
[2]Garten: Tigerstedt: Handbuch der Physiol. Methodik, 1911, i, 103.
[3]Frank: Zeitschr. Biol., 1913, xlvi, 519.
[4]Tigerstedt, C.: Skan. Arch. f. Physiol., 1913, xxviii, 37.
[5]Straub: Deutsch. Arch. f. klin. Med., 1914, cxiii, 535.

A NEW UNIVERSAL OPTICAL MANOMETER*

By Carl J. Wiggers, M.D., and William R. Baker, Cleveland, Ohio

IT is a relatively simple matter to construct an optically recording manometer which is sufficiently sensitive to record the details of intraventricular and aortic pressure changes and still has the requisite inherent vibration frequency to reproduce them with accuracy. It is a far more difficult task, however, to design the instrument so that it is satisfactory in experimental work. To do this the anatomic difficulties in its application, the proper alignment in relation to illuminating lamps and camera and a certain flexibility in adjusting the reflected beams horizontally as well as vertically have to be taken into consideration, not to mention the necessity of maintaining an adequate "light value" in the reflected beams.

In 1914, one of us[1] described a form of optical manometer which represented so great a technical advantage over existing forms that it has proved the instrument of choice in our laboratory ever since. In spite of several minor changes leading to simplification this instrument still has certain disadvantages which not only necessitate a certain amount of skill in its operation but which also somewhat restrict its uses.

Briefly these disadvantages are as follows:

1. In order to obtain a vertical and horizontal adjustment of the reflected beam, a second tilted and rotating mirror was employed. As a first surface mirror is not feasible (owing to accidental sprinkling with blood and solutions) the intensity of the reflected beam is so greatly reduced that good records cannot be obtained at paper speeds greater than eighty mm. per second. On the other hand, on slow paper and on film, good results are often frustrated by the reflection of double images (i.e., both from the front and silvered surfaces of the mirror).

2. The manometer can only be used with photokymographs having a vertical slot and horizontally moving paper, a disadvantage because the cameras available commercially have all been developed in connection with string galvanometers and therefore have horizontal slots and a vertical movement of the paper or film.

3. Inasmuch as the manometer cannot be tilted far from a vertical position after insertion and as the segment surface cannot be altered independently of the entire manometer, it is technically difficult and at times impossible to place manometers close enough together to record pressures from adjacent points in the cardiovascular system. This also prevents their illumination by a single source of light such as described by Katz and Baker thus entailing the necessity of making careful and repeated checks as to possible errors of parallax.

The new model obviates all of these disadvantages, is easier to operate successfully and not only yields better contrast in records at similar speeds but can be used with bromide paper traveling up to 150 mm. per second.

As shown in Fig. 1, the apparatus consists of a vertical glass tube 1.6 cm. in diameter and 8 cm. long, closed above by a stopcock and bulb. To the stem of the latter a calibrating manometer may be attached and through this avenue a small quantity of anticoagulation fluid may also be introduced in order to prevent or dislodge accidental clots in the cannula. At the bottom, the standard cannula fittings described in the older form are attached. The segment capsule fixture is attached to a short lateral T-tube 4 cm. from the bottom of the glass manometer tube.

To this is attached the metal fixture providing for a movable segment, capsule surface (A), 4 mm. in diameter. This fixture is constructed as fol-
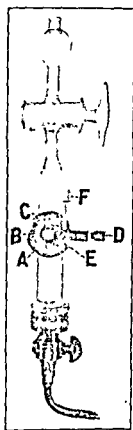

Fig 1.

lows: The segment capsule with a groove for fastening the rubber dam is fitted to a hollow brass ball (B). This is accurately ground to make a ball and socket joint with the metal attachment cemented to the short lateral glass tube and is firmly held in place by a lock ring (C). By means of this swivel joint, the entire capsule and its mirror can be adjusted in any direction by a convenient handle (D). Not only does this permit slight shifting of the light beams reflected to the camera slot, thus dispensing entirely with a second mirror arrangement, but by turning the ball through an angle of 90° the chord side can be swung to the right and the apparatus used with vertical beams and with cameras in which the paper moves vertically. The advantage of this arrangement in avoiding errors of parallax has been emphasized in a preceding paper by Katz and Baker.

In order to project a base line a second small mirror is carried on a small platelet (E). This is connected with the hollow ball (B) and segment

capsule in such a way that the mirrors attached to the rubber of the segment capsule and the platelet respectively, move together as the ball is rotated by the handle (*D*). Independent adjustment of the "base line mirror" is also possible, however, by another but smaller swivel joint controlled by a handle (*F*). When final adjustments have been completed the large ball is securely locked by tightening the ring (*C*) thus making disarrangement during the course of an experiment impossible.

The manometer has been tested practically during the past year in a diverse series of experiments, both with simpler forms of arc-lamps and with the double-slit mechanism described by Katz and Baker. These tests have demonstrated that this instrument contributes greatly to simplify the technic of optical registrations of dynamic phenomena of the heart and circulation.

REFERENCE

[1] Wiggers: Am. Jour. Physiol., 1914, xxxiii, 382.

THE IMPROVED NEUBAUER RULING FOR BLOOD CELL COUNTING*

By Simeon Trenner, Philadelphia, Pa.

THE Neubauer ruling has now generally supplanted the various rulings hitherto used in hemacytometry, as it combines all the advantages of the original Thoma, with its cell depth of 0.1 mm. and its central counting area of one square millimeter subdivided into 400 small squares for counting red cells, with an additional area of eight square millimeters, the four corner squares of which are subdivided into sixteen small squares to facilitate the counting of white cells.

For many years it was the custom to count the red cells in either the Thoma or Neubauer rulings in groups of twenty-five $\frac{1}{400}$ mm. squares, but it is now the almost universal practice to count these cells in groups of sixteen squares. This method saves time and insures greater accuracy as no computation is required, it being only necessary, with a dilution of 1:200, to add four ciphers to the count of eighty small squares.

To meet the wishes of the advocates of both methods, and possibly because of manufacturing difficulties at that time, the small squares were divided in the Thoma and in the original Neubauer rulings into groups of both sixteen and twenty-five by the addition of an extra line in the middle of every fifth square (see Figs. 1 and 1-*A*).

The need for a simple boundary occupying less space than this and less confusing in use was suggested in a personal communication by Roger S. Morris, M.D., University of Cincinnati. This improvement, based upon the suggestion of Morris and now embodied in the improved Neubauer ruling, consists in the division of the 400 small squares comprising the central square millimeter into twenty-five groups of sixteen by a new "split" line.

This appears under the microscope as a transparent boundary line for each group of sixteen small squares (see Figs. 2 and 2-A) and, since the confusing line through the middle of each fifth square has been eliminated, the area of the entire 400 squares is available for counting instead of 256 as in the original Neubauer.

The counting of cells, with a 16 mm. objective, in groups of sixteen small squares instead of in larger groups of twenty-five squares *permits the use of the center of the field.*

Some of the *advantages of this improvement in ruling,* in combination with the technic recommended for its use, are as follows:
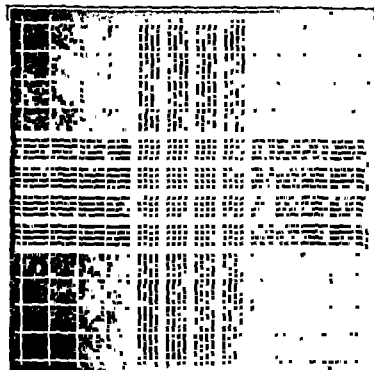

Fig. 1.—Entire area of *original* Neubauer ruling in which the groups of 16 smallest squares are separated by an extra line in the middle of every fifth square.
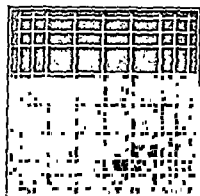
Fig. 1-A.—Group of 16 smallest squares of *original* Neubauer ruling showing boundary by fifth squares with extra line in middle of each.

There is no confusion in orienting and grouping the lines and cells in counting, as the worker passes from group to group with ease and certainty.

There is less eye strain in continuous counting as the field is small, flat, and evenly illuminated and the observer is not compelled to keep his eye close to the ocular or rigidly in the optical axis.

Continuous manipulation of the fine adjustment is unnecessary as the ruling is in sharp contrast to the extreme corners of the group and all the cells and squares are in perfect focus simultaneously.

Higher eyepiecing is possible without removal of spectacles than when a larger group of twenty-five squares is counted. *"Sweating" of the eye lens and the resultant loss of definition is to a great extent prevented.*

The draw tube can be extended for increased magnification when necessary without undue distortion or breaking down of the image.

Photographic records can be made with a 16 mm. achromatic, 0.65 N.A., objective that are sharp to the corners of the group.

The technic of counting is quickly conveyed by the demonstrator and grasped by the student.

The entire central square millimeter is conveniently visible in the field of the 16 mm. objective, just as in the Thoma and original Neubauer rulings.

The four corner square millimeters are, as in the original Neubauer, each subdivided into sixteen squares for convenience in counting white cells.

The improvement in ruling involves no basic change in the usual counting technic, as the same subdivisions of the nine square millimeters of counting area of the original Neubauer ruling, are retained.

With the improved Neubauer ruling the count is made from the center of the white or transparent line—visible as a result of the splitting of the line bordering each group of sixteen small squares—to the next engraved line when counting the cells in one square, or to the center of the next transparent line when counting the cells in a group of sixteen squares, employing the usual rule regarding cells touching the lines on two adjacent sides.

If the worker prefers metric computation by counting in groups of twenty-five squares, this can be accomplished by counting the cells in the nine small squares bordering on two adjacent sides of the split boundary
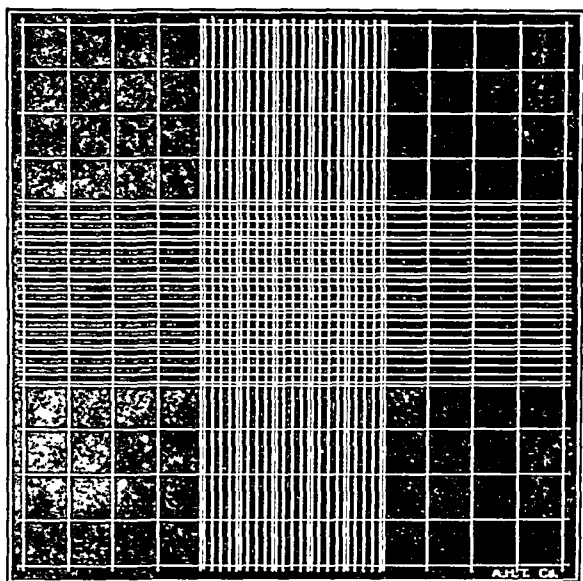

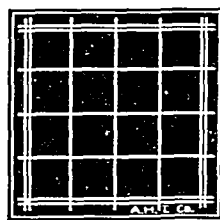Fig. 2.—Entire area of *improved* Neubauer ruling, showing split boundary lines.

Fig. 2-*A*.—Group of 16 smallest squares of *improved* Neubauer ruling showing split boundary lines.

line of the group of sixteen squares. The counting of four such groups widely separated gives the same metric red cell counting unit as in the Thoma and original Neubauer rulings. The same result is also attained by counting the cells in five rows of twenty small squares each horizontally across the field.

Cells can be counted by any of the several methods in general use; i.e., horizontally across the field, diagonally, or the center and corner squares, with increased facility because of the absence of the confusing extra line in every fifth square.

When the greatest possible accuracy is desired, a double chamber is used, with a drop from each of two pipettes, and the cells counted in the entire area of both rulings. This can be done with greater convenience than with the original Neubauer and Thoma rulings as there is no bisection of the peripheral rectangles in the improved Neubauer ruling.

QUANTITATIVE METHODS FOR THE DETERMINATION OF FORMIC ACID IN BLOOD AND URINE*

By F. De Eds, San Francisco, Cal.

METHODS for the determination of small quantities of formic acid in blood and urine are essential in studies of the fate of formaldehyde, hexamethylenamine, and methyl alcohol in the organism. Such methods should permit the determination of very small quantities of formic acid in order to ascertain with a reasonable degree of certainty the possible occurrence of formaldehyde in low antiseptic concentrations from the hydrolysis of hexamethylenamine, and the possible conversion of methyl alcohol to formic acid. Methods answering these requirements are not available in the literature, and the methods that have been used are not only not sensitive enough, but also inaccurate, thus rendering our present knowledge of the fate of formaldehyde and its compounds, and of methyl alcohol insecure, and a reinvestigation of the entire subject desirable.

The object of this paper is to demonstrate the unreliability of the methods of Pohl,[1] and Autenrieth[2] for the determination of formic acid in urine, and to propose methods which are applicable to blood and urine for the determination of such small quantities of formic acid as 0.12 mg. in 25 c.c. of blood, and 1.2 mg. in 150 c.c. of urine, corresponding to about 1:200,000 and 1:125,000, respectively. Before proceeding to a description of the proposed methods, the older methods will be discussed.

OLDER METHODS

The principal quantitative methods for formic acid described in the literature depend upon the reduction of salts of silver, or of mercury, the product of reduction then being estimated by gravimetric or volumetric processes. The reduction of mercuric chloride to calomel is typical of these methods.

1. Method of Autenrieth for Urine.—Three hundred c.c. of urine are treated with 30 c.c. of 25 per cent phosphoric acid, and placed in a 750 c c. Jena glass flask with a few pieces of broken porcelain. The flask is connected with a condenser for direct distillation. Heating is started with a small flame to prevent frothing, the intensity of the heating being gradually increased. Three hundred c.c. of distillate are collected in a graduated cylinder. Distillation is stopped, 300 c.c. of water added to the residue and distillation resumed. This process is repeated until the distillate is no longer acid, this point being reached when a total of 1,400 to 1,500 c.c. of the distillate are obtained. The combined distillates are now treated with an excess of calcium carbonate, and evaporated on

using starch as indicator in the usual way. The number of cubic centimeters of N/10 iodine required multiplied by 0.0023 represented the amount of formic acid in the volume taken for analysis.

A great many trials were made with this method without obtaining satisfactory results. Successive determinations on the same blood failed to give check results. In most instances, metallic mercury in varying amounts was one of the products of reduction, and in the presence of metallic mercury the thiosulphate titrations are obviously meaningless. However, the titration feature of the method was recognized as desirable and was made a part of the method to be proposed. The chief difficulty with blood was to obtain a filtrate free from reducing substances other than formic acid.

Various methods of obtaining such a blood filtrate were tried. Precipitation of the protein with 95 per cent alcohol, with absolute alcohol, with absolute alcohol followed by the addition of a saturated alcoholic solution of zinc chloride proved inadequate when the modified Stepp and Zumbusch procedure was applied to the filtrates. Check determinations were not obtained, and reduction of mercuric chloride to metallic mercury usually occurred. Finally, it was found that treatment of the blood with picric acid completely eliminated the difficulties encountered with other precipitants, and permitted the estimation of 0.1 mg. of formic acid in 25 c.c. of whole blood. The following procedure was adopted for blood.

PROPOSED METHODS

1. *Blood.*—The sample of blood (10 c.c. to 25 c.c.) drawn into a pipette moistened with saturated oxalate is placed in a glass stoppered cylinder of 100 c.c. capacity and diluted to 50 or 60 c.c. with distilled water. The cylinder is stoppered and inverted several times to insure good mixing and hemolysis. After removing and washing the stopper carefully with a few cubic centimeters of water, 5 gm. of picric acid are added. The cylinder is gently rotated until the picric acid sinks. The sides of the cylinder are then washed down with distilled water and the volume made up to the 100 c.c. mark. The cylinder is now stoppered and shaken vigorously until any emulsion formed is broken up, and there is no further change in color. The final color should be a muddy yellow. The material is thrown upon a large filter, and the filtrate collected in a 100 c.c. cylindrical graduate. Filtration is continued until dripping from the funnel ceases. A clear yellow filtrate results. Fifty c.c. of the filtrate are transferred to a 300 c.c. Florence flask, and 5.0 c.c. of mercuric chloride reagent added. The composition of this reagent is the same as that of Fincke[6] and consists of the following ingredients: mercuric chloride 10 per cent, sodium chloride 10 per cent, and sodium acetate 15 per cent in distilled water. Then the mixture is heated on the water-bath for 2 hours and the amount of calomel formed is determined volumetrically as follows: The flask is cooled in running water, and 10 c.c. of 25 per cent hydrochloric acid are added, followed by 4 gm. of potassium iodide and 10 c.c. of N/10 iodine solution. The flask is rotated until all the calomel disappears, by conversion to the soluble mercuric chlo-

ride. The excess iodine is then titrated with N/10 sodium thiosulphate from a microburette of 5 c.c. capacity, using starch as indicator. The end point is the change from green to yellow, and is quite sharp by daylight, less so by artificial light. The calculation is as follows:

c.c. of N/10 iodine x 2 x 0.0023 = gm. formic acid per volume of blood taken.

This method was applied to horse serum, whole beef blood, and whole dog blood before and after the addition of known amounts of formic acid. Typical data are presented in Table II.

TABLE II

DETERMINATION OF FORMIC ACID IN BLOOD AND SERUM

BLOOD OR SERUM (25 c.c.)	FORMIC ACID ADDED	FORMIC ACID FOUND	PER CENT RECOVERY
	gm.	gm.	
Whole Beef Blood	none	none	none
Whole Beef Blood	0.00470	0.0055	117
Whole Beef Blood	0.00115	0.00137	114
Whole Beef Blood	0.00012	0.00013	108
Horse Serum (preserved)	none	none	none
Horse Serum (preserved)	0.00115	0.00115	100
Horse Serum (preserved)	0.00115	0.00111	96.6
Horse Serum (preserved)	0.00230	0.00230	100
Horse Serum (preserved)	0.00230	0.00229	100
Whole Dog Blood	0.00050	0.00050	100
Whole Dog Blood	0.00012	0.00011	91.7

It is seen that amounts of formic acid as low as 0.00012 gm. in 25 c.c. of whole blood or serum can be satisfactorily determined, or in other words, a concentration of about 1:200,000. The blood and serum blanks gave no values for formic acid, indicating that picric acid effects a complete removal of reducing substances other than formic acid in these fluids. Accordingly, the sensitivity and accuracy is great enough for all purposes necessary in studies on the distribution of formate, and the fate of formaldehyde and its compounds, and of methyl alcohol in the organism. However, before the method could be applied in such studies it was necessary to ascertain to what extent these agents will give positive results with the proposed method and whether or not they can be removed. Interference from formaldehyde and hexamethylenamine was expected, but not from methyl alcohol. The latter expectation was fully confirmed.

APPLICATION OF THE METHOD IN PRESENCE OF METHYL ALCOHOL, FORMALDEHYDE AND HEXAMETHYLENAMINE

Methyl alcohol was boiled with mercuric chloride reagent, and gave no production of calomel. Formaldehyde yielded calomel, and likewise hexamethylenamine, since heating facilitated hydrolysis of hexamethylenamine with the liberation of formaldehyde. However, hexamethylenamine was quantitatively precipitated by picric acid and so removed. Formaldehyde was removed by adding 1 c.c. of 10 per cent ammonia to the blood before the addition of the picric acid. Hexamethylenamine was thus formed and precipitated upon the addition of the picric acid. Confirmatory evidence for

the effectiveness of these steps was obtained by the following experiments: One-tenth gram of hexamethylenamine was added to 25 c.c. of whole beef blood, picric acid added, and the analysis carried out as described above. No formic acid was found. A second 25 c.c. portion of the same blood was treated with 0.2 c.c. of 40 per cent formaldehyde and 1 c.c. of 10 per cent ammonia. No formic acid was found upon analysis. A third portion was treated with 0.2 c.c. of 40 per cent formaldehyde, 1 c.c. of 10 per cent ammonia, and 0.0005 gm. of formic acid added. Analysis showed the presence of 0.00051 gm. of formic acid, or, in other words, complete removal of the formaldehyde.

2. *Urine.*—During the trials with the methods of Autenrieth and Pohl on urines, the observation was repeatedly made that reduction of mercuric chloride frequently went to the stage of metallic mercury. Later it was observed that this reduction to metallic mercury was greater, the greater the creatinine content. Having noted this, urea, uric acid and creatinine potassium picrate were investigated for their reducing action by boiling with the mercuric chloride reagent. Out of all these substances creatinine potassium picrate was the only one which caused reduction of mercuric chloride, and the reduction went to the stage of metallic mercury. Consequently, the removal of creatinine from urine was sought for, by making use of the principle found to be successful for analysis of blood, that is, by precipitation with picric acid, and as follows:

One hundred to 150 c.c. freshly voided and 25 c.c. portions of concentrated urines were treated with 5 gm. of picric acid, and shaken vigorously for different time periods. The details of numerous attempts to standardize the time of shaking and process of filtering will be omitted. In brief, the removal of creatinine as the picrate was not uniformly quantitative. Therefore, steam distillation of the picrate urine mixture was resorted to and the following procedure adopted.

One hundred to 150 c.c. of *fresh* urine are placed in a one liter Erlenmeyer flask, 5 gm. of picric acid added and the flask rotated vigorously for ten minutes. The flask is then connected for steam distillation and a distillate of not less than 2000 c.c. collected, 1 c.c. of 20 per cent sodium carbonate being placed in the receiving flask in order to neutralize the formic acid distilled over. The distillate is then transferred to a large evaporating dish and evaporated to about 100 c.c., preferably on a water-bath. The concentrated distillate is then transferred to a Florence flask of 500 c.c. capacity and made slightly acid to litmus with a few drops of 25 per cent hydrochloric acid. Then 5 c.c. of the mercuric chloride reagent used in the blood method are added. The rest of the procedure is the same as in the method proposed for blood and described above. Titration of the calomel is used since it is less troublesome than the gravimetric method. This method was applied to twelve urines. Typical results are presented in Table III.

Table III shows that 1.2 mg. of formic acid added to 150 c.c. of fresh urine may be satisfactorily determined. Since urine to which no formic acid was added gave no formic acid values, and since the principle of precipita-

TABLE III

DETERMINATION OF FORMIC ACID ADDED TO URINE

URINE	FORMIC ACID ADDED	FORMIC ACID FOUND	PER CENT RECOVERED
	gm.	gm.	
100 c.c. fresh urine alone	none	none	none
100 c.c. fresh urine containing formic acid	0.00405	0 00412	101
100 c.c. fresh urine containing formic acid	0.00405	0.00395	97.5
100 c.c. fresh urine containing formic acid	0 00405	0.00360	88.8
150 c.c. of another urine	none	none	none
150 c.c. same urine containing formic acid	0.0040	0 00400	100
150 c.c. same urine containing formic acid	0.0020	0 00210	105
150 c.c. same urine containing formic acid	0.0081	0.0080	98 7
150 c.c. same urine containing formic acid	0.0012	0.00115	95.8

tion with picric acid was used, it seems logical to conclude that the variable results, obtained with the older methods in the first part of the paper, are due to creatinine carried over in variable amounts during distillation.

USE OF PRESERVATIVES

The use of fresh urine is necessary because old urines were found to give definite values when no formic acid had been added. When working with old urines the reduction of mercuric chloride did not go to metallic mercury, hence the reduction was caused by something other than creatinine. Bacterial decomposition may have yielded reducing substances not removed by the picric acid. McGuigan[7] claims that on standing the formic acid content of urine is increased. However, results obtained on urine preserved with chloroform showed that this increase in formic acid on standing urines may be prevented. This matter was tested as follows: A sample of urine was divided into three equal portions, A, B, and C. A was analyzed fresh, no formic acid being added. B was preserved with chloroform and analyzed two days later. C was analyzed at the same time as B, but had not been

TABLE VI

TITRATION VALUES FOR FORMIC ACID IN FRESH, PRESERVED AND STANDING URINES

URINE OR SOLUTION	N/10 SODIUM THIOSULPHATE C C. REQUIRED	FORMIC ACID VALUES; AND REMARKS
10 c.c. Iodine	10.10	
A. Fresh urine	10.11	none; no calomel
B. Urine preserved 2 days with CHCl₃	10.09	none; no calomel
C. Urine standing 2 days without CHCl₃	8.94	0.0026 gm ; calomel present

preserved with chloroform. The titration results obtained are presented in Table IV, and are self-explanatory, indicating that no formic acid was found, except in the unpreserved urine. Chloroform, therefore, was a suitable preservative.

The use of thymol, cresol, formaldehyde, etc., as preservatives is contraindicated because of a reducing action on mercuric chloride.

DISCUSSION

Autenrieth regarded the values obtained on normal urine by his method as due to a formic acid content. Pohl obtained values on normal urine but not of the same magnitude as those found by Autenrieth. Sollmann reported a large variation in formic acid content of the urine. Using the picric acid method proposed in this paper, a formic acid value has never been obtained on fresh urine, nor on urine preserved for two days with chloroform. It is, therefore, possible that the values obtained by the older methods are due to creatinine carried over during distillation. The same applies to the original method of Stepp and Zumbusch for blood. These authors report formic acid in normal blood. Here again I have found no production of calomel in normal blood when the picric acid method was used, thus giving further support to the possibility of the values being due to creatinine.

CONCLUSIONS

1. It was found that the small quantities of formic acid in urine and blood cannot be determined satisfactorily by the older methods of Pohl and Autenrieth (urine), and by a modification of the Stepp and Zumbusch method (blood).

2. Methods are proposed with which it is possible to determine satisfactorily such small quantities of formic acid as 0.12 mg. in 25 c.c. of blood, and 1.2 mg. in 150 c.c. of urine.

Thanks are due to Dr. P. J. Hanzlik for criticisms and suggestions during the work.

REFERENCES

[1]Pohl: Arch. f. exper. Path. u. Pharm., 1893, xxxi, 281.
[2]Autenrieth: Münch. med. Wchnschr., 1919, lxvi, 862.
[3]Sollmann: Jour. Am. Med. Assn., 1908, li, 818.
[4]Stepp and Zumbusch: Arch. klin. Med., 1920, cxxxiv, 112.
[5]Riesser: Zeit. physiol. Chem., 1915, xcvi, 355.
[6]Fincke: Zeit. nähr. Genussem, 1911, xxi, 1.
[7]McGuigan: Jour. Am. Med. Assn., 1914, lxii, 984.

A NEW CLINICAL METHOD FOR BLOOD TYPING*

By O. M. GRUHZIT, M.S., M.D., AND L. T. CLARK, B.S., DETROIT, MICH.

THE need for blood typing has rapidly extended from large hospital centers to the most distant obscure corners of the earth. Its usefulness has increased manifold from Landsteiner's[1] scientific study of heredity of blood characters to practice of blood transfusion for mechanical, toxemic or idiopathic exsanguination. Further, blood group properties are used for phylogenetic study of races.[2] Ottenberg[3] employs blood group characters in identification of parentage of a child. In surgery, Mason[4] introduced blood typing

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for selecting proper donors for skin grafting. Lastly, Gruhzit[5] introduced blood typing for differentiation of eclampsia from nephritic toxemias.

Irrespective of the wide use of blood typing, the methods employed have remained in general the same since the time of Landsteiner's (1901) macroscopic and Brem's[6] (1916) microscopic modification and has been strictly delegated to trained laboratory technicians.

The methods in vogue are time-consuming, expensive, and confined to laboratory workers. The present wide use of blood typing demands a method which could be employed by the general practitioners as well as trained technicians and would save time and expense. With this in view, the authors have devised a capillary tube with a central, spindle bulb, one end of which contains the serum, as shown in Fig. 1, and which embodies the above requirements.

THE METHOD

The bent end of the capillary is filled with a standard serum prepared and standardized in the usual manner. It has been our accepted custom to avoid
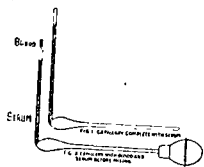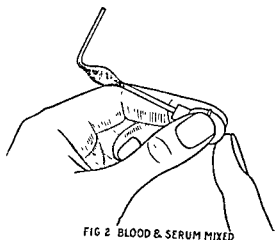

Fig. 1.

FIG 2 BLOOD & SERUM MIXED

mixing of sera, to put Serum II in flint glass and Serum III in amber glass capillaries.

Before puncturing the patient's finger or ear, break off the tip of the empty end of capillary, then break off the tip of the end containing serum. This order is essential, as it avoids splashing of serum. Place a small rubber bulb on the empty end of the capillary. Prepare the other capillary in a similar manner. Prick the patient's finger or ear, previously cleansed with alcohol, with a sharp, sterile pin. Squeeze out a minute drop of blood and touch it for an instant with the end of the capillary containing serum. Repeat this with the other tube.

Now grasp the rubber bulb between the left hand thumb and index finger. Place the tip of the right index finger upon the opening in the rubber bulb as shown in Fig. 2. Gradually release the left hand fingers. This draws cells and serum into the spindle enlargement of the capillary. Repeat the compression and release several times. This mixes the cells and serum thoroughly. Repeat this with the second tube.

If the results are read at the place of taking the sample, the capillaries

are set aside for about five to ten minutes, then reagitated and results are noted.

If capillaries are transported a small cork is placed over the tip to avoid spilling.

READING OF RESULTS

Before reading results reagitate contents. If the contents appear granular on agitation and if the flocculation increases on agitation, it means that there is agglutination. Mixture that remains uniform without distinct flocculation to naked eyes, is negative or shows no agglutination.

If it is desired to check up the macroscopic reading with a microscopic, the contents are thrown on plain slide and examined under microscope.

To collect the blood usually takes less than a minute. To read results usually takes five minutes. The test may be completed in five to ten minutes.

There is only one precaution—and that is not to take more of the patient's blood than a mere trace. In fact one never can have too little of blood.

ADVANTAGES OF THE NEW METHOD

The test can be performed by any physician at any place irrespective of laboratory facilities.

The test is simple; results are plainly visible.

The test combines the various steps of the older technic into one.

REFERENCES

[1]Landsteiner: Wien. klin Wchnschr., 1901, xiv, 1132.
[2]Fukamachi, H.:   Jour. Immunol., 1923, viii, 291.
[3]Ottenberg, R.:   Jour. Am. Med. Assn., 1920, lxxviii, 883.
[4]Mason, J. C.:   Jour. Am. Med. Assn., 1918, lxx, 1581.
[5]Gruhzit, O. M.:   Am. Jour. Obst. and Gynec., April, 1923, v, 400.
[6]Brem, W. V.:   Jour. Am. Med. Assn., 1916, lxvii, 190.

TRANSACTIONS

THIRD ANNUAL CONVENTION AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, ROCHESTER, MINNESOTA

June 5, 1924

The members assembled at the registration desk on the sixth floor of the surgical pavilion, St. Mary's Hospital, Rochester, Minnesota at 8:00 A. M., June 5, 1924. At this desk they were registered and given cards of admission to all of the laboratories and surgical clinics of the Mayo Foundation. As they completed their registration, the members distributed themselves to the laboratories in which they were most interested, particularly the Pathological Department conducted at St. Mary's Hospital. At noon on this day the members were invited by Dr. and Mrs. William J. Mayo to luncheon at their residence. This proved to be a very enjoyable event, and gave members an opportunity to meet each other in a social atmosphere.

At 2.00 P. M. the meeting was called to order in the Assembly Room of the Mayo Clinic and the scientific program began. The following papers were then read:

"Studies in Pollen Sensitization"—J. H. Black, M.D., Dallas, Texas. There was no discussion of this paper.

"A Quantitative Wassermann Test"—E. H. Ruediger, M.D., Los Angeles, California. This paper was discussed by John A. Kolmer, M.D., Philadelphia, Pa.; A. H. Sanford, M.D., Mayo Clinic, Rochester, Minnesota; H. G. Marquez, M.D., San Francisco, Calif.; E. H. Ruediger, M.D. made a few closing remarks relative to his paper.

"Odd Laboratory Appliances"—Francis L. Burnett, M.D., Boston, Mass. No discussion.

"Notes on the Clinical Examination of Protein in Urine with Description of Simple and Rapid Method"—William G. Exton, M D, New York City. There was no discussion, although several questions were asked of Dr. Exton regarding his paper.

"A Moving Picture Demonstration of the Theory and Practice of the Wassermann Reaction"—C. E. Roderick, M.D., Battle Creek, Mich. No discussion followed.

"Some Physico-Chemical Aspects of Hemolysis"—E. C. Mason, M.D., and P. R. Rockwood, M.D. (By invitation.) This paper was discussed by John A. Kolmer, M.D., and P. R. Rockwood, M.D., also by Drs. Castleman and Brown. The discussions, as well as the papers, were most lucid and interesting. The exchange of views and ideas on the different topics which the papers presented were of unusual value and significance.

There being no further business the meeting adjourned.

President MacCarty called the meeting to order at 8.00 P. M. in the Assembly Room of the Mayo Clinic, a joint meeting with the American Society of Thoracic Surgeons.

Dr. Harry J. Corper of Denver talked on "Personal Experiences with the Cultivation of Tubercle Bacilli and the Use of the Guinea Pig as a Diagnostic Test Animal for Tuberculosis."

Dr. E. C. Kendall, of Rochester, gave the "History of the Discovery of Thyroxin," while Dr. J. G. Hardenbergh, also of Rochester, spoke on the "Care of Experimental Animals." These addresses proved to be most profitable as well as interesting.

The meeting was then adjourned.

June 6, 1924

On Friday, June 6, the meeting was called to order at 8.00 A. M. in the Assembly Room of the Mayo Clinic by President MacCarty.

First, there was a Round Table discussion of the clinical value of laboratory procedures, after which there were discussions on the following topics:

"General Clinical Laboratories" was thoroughly discussed by Dr. A. H. Sanford.

Dr. L. G. Rowntree, spoke on "Medical Laboratory Problems."

"Metabolic Laboratories" was the subject of Dr. W. M. Boothby's talk.

"Diabetic Laboratories" was discussed by Dr. R. M. Wilder.

Dr. H. F. Helmholz gave a discussion on "Pediatric Laboratories."

"Surgical Diagnostic Laboratories" was the subject of Dr. A. C. Broders' discussion. Each of these discussions was genuinely appreciated and enthusiastically received by the members.

At 10.00 A. M. visits to the various diagnostic laboratories were made and the members dispersed until afternoon.

At 2.00 P. M. Dr. MacCarty called the meeting to order in the Assembly Room of the Mayo Clinic.

The following papers were read:

"Teaching of Clinical Pathology in the Medical Schools" by Dr. Francis B. Johnson, Charleston, S. C. No discussion.

"A Study of the Specificity of the Kolmer Complement-Fixation Test for Syphilis" by Dr. John A. Kolmer, Philadelphia, Pa. This paper was discussed by Dr. A. H. Sanford, Rochester, Minnesota; Dr. George Ives, St. Louis, Missouri; Dr. E. H. Ruediger, Los Angeles, California; Dr. J. V. Falisi, Fitzsimons Hospital, Denver, Colorado. Dr. Kolmer closed the discussion.

"Several Phases of Spinous Cell Carcinoma" by L. A. Turley, M.A., Ph.D., of Norman, Oklahoma. This paper was discussed by Dr. Philip Hillkowitz of Denver, Colorado. Dr. Turley made the closing remarks.

"Better Postmortem Service" by Dr. H. E. Robertson, M.D., Rochester, Minnesota. (By invitation.) Dr. John A. Kolmer, Philadephia, Pa., and Dr. H. E. Brown, Rochester, N. Y., discussed this paper. Dr. Robertson closed the discussion.

"Interpretation of the More Practical Blood Examinations in Tuberculosis" by Dr. H. G. Sweany, Chicago, Ill. This paper was discussed by Dr. Harry J. Corper of Denver.

"The Practical Value of Examinations of the Saliva in Urea Determination by Dr. P. S. Hench, Rochester, Minn. (By invitation.) Dr. W. G. Exton discussed this paper.

One was aware of the unselfish devotion to their profession of those who, by their long experience, were enabled to submit these various papers for our consideration. The earnestness and zeal with which the papers were discussed was also felt and appreciated.

At 7:00 P. M. the Clinical Pathologists and their wives gathered together for the Banquet at the Kahler Hotel. It is superfluous to add that it was a most charming and entertaining affair.

Not least among the pleasures of the evening were the addresses of Dr. William C. MacCarty, Dr. William J. Mayo and Dr. Leonard Freeman.

Dr. L. B. Wilson delivered an address on "Graduate Education in Clinical Laboratory Branches of Medicine."

Dr. M. T. MacEachern gave an address on "The Clinical Pathologist and the American College of Surgeons."

A feeling of close comradeship was developed and forged at the various gatherings that will prove unforgettable and enduring.

June 7, 1924

Saturday, June 7 at 8:00 A. M. Dr. MacCarty called the meeting to order in the Assembly Room of the Mayo Clinic.

The scientific program commenced. Due to the absence of the authors the following papers were read by title:

"The Influence of the Natural Anti-Sheep Hemolysins of Human Sera Upon the Production of Anomalous Reactions in the First Tube of Kolmer's Quantitative Complement-Fixation Test for Syphilis."—Robert A. Kilduffe, M.D., Los Angeles, Calif.

"Preparation of Jaundiced Patients for Operation." by Waltman Walters, M.D., Rochester, Minn. (By invitation.)

"Pre-cancerous Conditions."—O. J. West, M.D., Seattle, Wash.

"Notes on Treatment of Nasal Diphtheria Carriers."—Clara Israeli, M.D., Manchester, N. H.

Minutes of the previous meeting having been published in the Journal, reading of the same was waived. The President stated that it was not necessary to appoint new committees, and announced that there was no unfinished business.

The Executive Committee, acting as an Auditing Committee, certified the Secretary-Treasurer's books to be correct and their report to that effect was accepted.

The Board of Censors reported orally that they had investigated and approved fifty-one applications for membership and the report was accepted and applicants elected.

The Committee on Standardization of Reagents made no report.

The Committee on Nominations submitted the following:

For president, Dr. John A. Kolmer, Philadelphia, Pa.; for first vice-president, Dr. F. E. Sondern, New York City; second vice-president, Dr. W. F. Thomson, Beaumont, Texas; secretary-treasurer, Dr. Ward T. Burdick, Denver, Colorado.

Dr. Kolmer, upon being nominated for president, made a speech expressing his appreciation of the honor, and in apt terms spoke of the responsibilities the office involved, and of his hesitancy in undertaking the same should he be elected.

All the officers were elected by acclamation.

On motion, a rising vote of thanks was tendered to Dr. William Mayo and Mrs. William Mayo, the Board of Governors of the Mayo Foundation, the Executive Committee, the Ladies Committee and local Committee on Arrangements for the efforts which they put forth for the entertainment of the Society.

On motion the resolution from the American College of Surgeons was accepted.

It was moved to amend one of the by-laws so that it would read as follows: "The Society shall elect annually by ballot at an executive session held during the time of the annual convention," instead of reading, "held on the second day of the annual convention" which motion was carried.

On amended motion, the matter of preparing a leaflet conveying information and instructions on the manner in which specimens are to be sent to the laboratories for examination was referred to the Executive Committee with instructions to submit the same at the next annual meeting of the Society.

It was moved that the committee now in charge of the matter of the standardization of laboratories be continued and that they meet in considering that subject with Dr. L. B. Wilson. Motion carried.

It was moved that the Society present to Dr. L. B. Wilson and his committee, a statement to the effect that The American Society of Clinical Pathologists is considering the matter of laboratory standardization, approves of such course or action, and is still considering the best methods by which such object may be attained. Motion carried.

Dr. Hillkowitz, chairman of the Publishing Committee, spoke of the importance of having a Journal that would be interested in the labors and researches of this Society, and that Dr. Warren T. Vaughan, Editor-in-Chief of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, had declared the Journal's intention to emphasize problems that are of practical expediency to clinical pathologists and to include in the editorial staff a greater number of members.

Dr. Burnett, in line with ideas expressed by Dr. Harry J Corper, suggested that the matter involving the best means of stimulating labor and research work among clinical pathologists, be referred to the Executive Committee for its attention. Motion carried.

It was moved that the place of the next meeting be left to the Executive Committee. Motion amended, designating Philadelphia as the meeting place.

Motion and amendment later withdrawn.

Remarks were made by retiring President MacCarty, who yielded the chair to the new president, Dr. Kolmer, who expressed his appreciation of the confidence reposed in him, and with a few well chosen words of encouragement and inspiration outlined in brief the purposes and main objects of the Society, and spoke in glowing terms of the indebtedness

to Doctors MacCarty, Sanford, Broders and others on the Committee on Arrangements and the Committee on Entertainment, of the Mayo Clinic for the splendid success of the Society's meeting. His views in that behalf met with the most enthusiastic applause.

On motion meeting adjourned until 2.00 P. M.

At 2:00 P. M. the members were conducted in automobiles to the Mayo Farm where they were shown the animal enclosures and various other interesting features.

At 4:00 P. M. there was a pleasant drive in the country which was participated in by the members.

A delightful picnic was held at the Rochester Country Club at 6:00 P. M. and thus ended the third annual convention of The American Society of Clinical Pathologists.

As an authority with a far-sighted vision there are few who will ever exceed Dr. W. J. Mayo, of Rochester. His words of inestimable value to the clinical pathologist, follow here as presented to the attending members the evening of June sixth. It is for those who were not so fortunate as to be able to hear Dr. Mayo, that this is published, as well as for a permanent record for all clinical pathologists.

Dr. W. J. Mayo.—As a general surgeon, I am glad to have this opportunity to express my obligation to the clinical pathologists, who have made possible one of the greatest advances in the surgery of the last decade. In the earlier day, the general surgeon, with the aid of the special senses, especially those of sight and touch, was engaged in gross surgery, usually of the destructive type, owing to the fact that the pathologic processes of which he was able to take cognizance were advanced and, as a rule, indicated radical treatment. Today, through the aid of the various clinical laboratories, the surgeon works with the eye of the microscope, if one may so speak. Pathologic processes are discovered in the early stages of development, with the result that operative procedures can be followed when deviation from the normal physiology is least gross, and surgery has become constructive in its general tendency.

The surgeon can be regarded as a means of mechanical therapy guided by the microscope, and for this guidance in the Clinic we are indebted to MacCarty and Broders and their associates. For example, on opening the abdominal cavity to operate on a neoplasm, a small piece of tissue may be removed for microscopic examination and, within two or three minutes, not only is the nature of the growth known, but also, by the aid of Broders' Index of malignancy, based on MacCarty's work on cell differentiation, the prognosis. If the cells are highly differentiated, the prospect of permanent cure is so great that one is justified in undertaking the serious type of operation, whereas, if the cells are largely undifferentiated, the prospect of cure is so small that a formidable operation would not be justified.

Again, as in carcinoma of the large intestine or rectum, when enlarged glands occur, instead of assuming that the condition is incurable, a gland may be removed for microscopic examination. Often, enlarged glands are the result of sepsis, in which case operation might possibly effect a cure.

Not only is the work of the clinical pathologist important in the operating room, but also in the diagnostic clinic. The diagnosis in a given case may be in doubt. Before starting the patient on a long series of indefinite laboratory examinations which he may be in poor condition to withstand, it is often possible, under local anesthesia, to take a specimen of tissue from an enlarged gland in the groin or supraclavicular fossa, a thickened umbilicus, or from some other indicated situation, to be subjected to the acid test of a microscopic examination which tells truly the nature of the trouble and indicates the proper treatment for it.

The future holds much for you gentlemen, in direct proportion to the recognition of your work by the public and its employment for the benefit of the patient by the medical man.

President MacCarty.—Those of you who know anything about what has happened in this Clinic in the last seventeen or eighteen years, know full well that no one man can know everything about any one subject. It makes no difference how small the subject

may be. In the very early experiences here, when we were trying to learn something about thyroids, occasionally we thought we knew something about them but we learned very soon that our knowledge was limited. We found out early that the only way we could investigate the subject in the Clinic was to have the coöperation of the chemists, physiologists, anatomists, and pathologists; this evening we want to hear from our chemist. This Clinic is very proud of him and his department, which plays a very important rôle in our institution. He hardly needs any introduction because the medical profession has been interested in his work for many years and knows of his splendid achievement in the separation of thyroxin. I thought it would be of interest to you to know something about the evolution of his experiences in connection with thyroxin; I want to present Dr. Kendall, who has charge of this interesting investigation.

Dr. Kendall (Rochester, Minnesota).—Most of you doubtless know of the isolation and identification of thyroxin, and I have not time to give you a recital of the work that has been going on for the past fourteen years. But there are certain details which have come out of the work which show what chemistry can do in a study of the ductless glands, and I should like to have you think of the relation of chemistry to a thing as involved and as far-reaching as the ductless glands. There are clinical classifications of the ductless glands, pathologic classifications and many other classifications, but no one approach to the subject can tell the whole story. There must be some underlying facts by which they are related, and I think that chemistry is going to tell more about that relation than any other science can tell us.

It has been said that universities are made up of persons and traditions, because if you take the traditions and persons away from a university, you have nothing left but the buildings, which in themselves are not of interest; and so it is with chemistry. Chemistry is made up of substances and ideas, but the ideas really are of more importance than the substances. After fourteen years of work in the study of the thyroid, we have a series of chemical substances, some of which are very suggestive and important, but besides that, we have a whole group of ideas, which start with thyroid and reach out through all the ductless glands.

The substance thyroxin was isolated in 1914 in pure crystalline form, and after two years we succeeded in preparing sufficient of it from the thyroid gland to start analyzing it, and finding out what it is chemically. In 1917, this was begun, and for two years we studied the chemistry of the molecule. In 1919 we stopped work with thyroxin itself, and began to try to make it synthetically from carbolic acid, ammonia, and things that are found in the chemical laboratory.

When they found out what adrenalin was, and wanted to make it synthetically, they went to the chemical supply house, and asked for three substances. Those three substances were put together in one molecule, which proved to be adrenalin. When we started out to make thyroxin synethetically, we could not buy three or thirty substances to make that molecule. Thyroxin belongs to a group of compounds which had not been made. For this reason we were forced to spend two years in studying these new compounds, picking up clues here and there, and finally evolving a series of steps which gave us a molecule which we thought should be thyroxin. Thyroxin contains 65 per cent of iodine which is attached to a substance which had never been made synthetically in the laboratory. The family of substances to which this belongs is a new contribution in organic chemistry. The method of the synthesis was to make the compound to which the iodine was attached, and then add the iodine to the molecule and see whether or not the resulting compound was thyroxin. We cheered ourselves up at this time by saying the iodine will go on easily after we get the molecule to which the iodine is attached; but that is not true. The iodine will not go on easily, and I believe that this fact is very significant in some of the disturbances in thyroid diseases. We have had trouble putting the iodine on, and I think the thyroid gland has trouble in putting iodine on. If the three atoms of iodine are not attached to the molecule, the molecule cannot function normally, and a pathologic condition will result.

I will point out some of the details in the work. Starting with the thyroid gland

itself, we boiled them in nickel kettles with sodium hydroxide. It was a very disagreeable operation; at least, the clinical department thought so, because this was done downstairs below the section on nervous diseases. After boiling with alkali we added acid, which threw out a small precipitate which contained the thyroxin that had not been destroyed. The gland contains about one part of thyroxin in ten thousand parts of gland. It is a very extravagant and expensive method to isolate it, and today one milligram costs thirty-five cents—three hundred dollars a gram. We hope synthetically to make it for about thirty cents a gram.

After isolating this substance, of course we were intensely interested in finding out its physiologic effec. A child ten years old with myxedema was treated with thyroxin. She grew four inches in six months, and has continued to develop. Between January 14, 1915, and January 30, 1919, she increased 81 per cent in weight and 43 per cent in height. Her improvement in mentality corresponds. When she came ten years ago she brought her doll with her. She was about the mental age of four, but she has since gone to school, and she is now nearly twenty-one years old.

After establishing the physiologic importance of thyroxin, we were most interested in the chemical reason for its activity. What was there in that molecule that had such a tremendous effect? There are only fifteen milligrams; that is, about one quarter of a grain, in the entire human adult, and yet that amount means the difference between a high grade myxedema and a normal human being. After establishing its formula, we sought the clue to its activity, but as long as we worked with thyroxin we did not uncover the secret. I doubt that we ever would have found how thyroxin works if we had used only thyroxin.

The chief reason for the attempt to synthesize thyroxin was to explain how it functions. We started in to make it synthetically, feeling sure that before we finished we should know all there was to be known about the molecule, because when it is divided up and then put together in different sections the effect of each section is easily seen. I am not going to bore you with how we went about synthesizing it, except to point out that the compound which we started out to make was to have a formula which we ourselves had already determined—that is to say, the compass for laying our course was made by ourselves, and if that compass eventually proved to be wrong, then practically all of our work would have been of no avail. For this reason we tested the intermediate products as they were secured, in order to determine which ones would possess physiologic activity. We found that all the compounds could be divided into three groups; those which produced immediate action; those possessing no physiologic action, and those which possessed a delayed but long-continued physiologic action. In all those possessing immediate physiologic action there was one arrangement of the molecule which was the same, and this brought out the fact these compounds can exist in two conditions, an oxidized form and a reduced form.

This brings up the question of physiologic activity of thyroxin in the animal organism. A few years ago, Wieland, of Germany, suggested that oxidation was not the building up of oxygen to a superoxidase, but that really the reverse took place, and hydrogen was first broken off. Some compounds were found which would take up hydrogen very readily. Now, we feel sure tonight that thyroxin is one of that group of substances. They are called hydrogen acceptors. They increase the rate of oxidation in the animal organism, acting as catalysers between the compounds to be burned and oxygen, and carrying hydrogen as an intermediate step. If the action of thyroxin lies in the taking up and breaking off of two atoms of hydrogen from the molecule, one might ask what good is the iodine? It is only recently that we have come to appreciate what the iodine in thyroxin does. The iodine is two-thirds of the weight of the molecule, and it dominates the activity of the entire molecule. It does not break off, but stays attached to three atoms of carbon. This is what we picture as the steps which occur when thyroxin functions. As it is put in the body, the pyrrol ring immediately opens. That we can prove in a test tube. Before it can act, the ring must close, and that is brought about by the physical state of the cell in which it functions. It does not close until forces act and close it, and those forces are acting through the nitrogen in the molecule. I cannot go into the details of that, but we know that it closes, and we know it must close before it can act. When it is closed,

the molecule can be oxidized and reduced, which produces a physiologic response in that particular cell. I shall not go any further into that theory, but we have a series of compounds which will prove or disprove that theory, and we have done enough to feel sure that those steps represent the way thyroxin really functions.

If we can feel sure that the function of the thyroid is to make hydrogen burn more easily, we have assigned a definite chemical action to one of the ductless glands, and it is the first gland which has been assigned a definite chemical action. Now, if we can say that the thyroid has to do with the burning of hydrogen, then with the same terminology, what does adrenalin do, what has the pituitary to do, and what has insulin to do? When these last questions are finally answered we will have a rational explanation of the function and mode of operation of the ductless glands, based on the series of chemical reactions initiated by their presence in the animal organism.

Doctor Rowntree.—As internists, our laboratories are our workshops. In them we attempt to determine the nature, and, by quantitative measurements, the degree of deviation from normal which occurs in disease. This leads us into many fields, such as physiology, chemistry, physics, and bacteriology, and clinical and scientific work become so intermingled that it is almost impossible to draw a line between them.

Dr. Sanford's talk has been extremely interesting to me. He has discussed the more important routine laboratory examinations and has given you his impressions based on wide experience. For many years, Dr. Sanford has had charge of all the routine laboratory procedures for a very large number of clinicians. I find his conclusions of great assistance in evaluating the various routine methods employed in medicine. Each of the tests discussed, and now considered routine, represents some individual investigation, and in reality, in considering routine tests, he discussed the successful researches of the last decade or two.

One great function of this institution is the training of men. The origin and development of the Mayo Foundation is interesting. Here we have two great practical surgeons, who with a number of associates, have developed a great institution which serves not only this part of the country, but the country as a whole. As they have grown older, they have experienced what so many have experienced in the past, the feeling that financial returns alone are not satisfying or wholly gratifying, and in recognition of the need for further progress in medicine, have established the Mayo Foundation for the promotion of medical education and research. As a result, we have here at the present time 175 graduate students, young men who have just finished their internship, and are seeking further training in medicine. These men are supported by the Mayo Foundation, which is generously endowed. This indicates how the minds of the practical surgeons may run to the support of medical education and research, and of scientific progress in medicine. The Foundation is a part of the University of Minnesota, and serves, as its name indicates, two great functions: medical education and medical research.

In the development of the medical side of this work I have been tremendously interested. It offers an exceptional opportunity. We have approximately fifty Fellows in medicine and the medical specialties. The training of these men is one of the primary functions of the institution. How are we going about their training? The first requisite, in most instances, is to make of these men good clinicians. If a physician is to be a good clinician, he must first become interested in sick people and their care. He usually obtains a good start along these lines during his internship, but still needs much additional training. As a rule, the first thing is to place him in the Clinic for clinical training on a modified apprentice system. On the floor of the Clinic, he obtains this in a way that is difficult to surpass. He may also see other phases of clinical work in some of the hospital services. As a rule, after a year or so of training, he comes to one of the hospital services, or he may select laboratory service. In certain hospital services the morning is devoted to the clinical side of the work, and a part or all of the afternoon to laboratory research. Broadly, we attempt to train the graduate student clinically, and at the same time to interest him in medical research in the Clinic, in the wards, and in the laboratories.

A graduate student, who has a particular problem in which he is interested, is always given the opportunity to work on it, provided this is feasible. Others ask for problems,

or may be assigned them by members of the permanent staff. Concerning the capacity of these men to handle research problems we have learned much. Generally speaking, for the first three months, the simpler the problem, and the more concrete, the better.

Certain considerations are fundamental to the development of clinical investigation. First, it is essential to have a problem that is definite; second, methods must be utilized that are capable of solving it; such methods may not exist, and so it may be necessary to devise one; third, adequate records must be kept; fourth, the results must be interpreted and correlated with existing knowledge. These seem very simple things. However, the majority of men starting investigation fail to grasp some or all of these simple principles.

Many men come to us with the idea of picking up crumbs from this or that service. They want to be spoon-fed, or to flit from flower to flower. As a rule, they accomplish little. In research, men of this sort are usually interested only in obtaining material for a thesis, so that they may have an advanced degree. Other men come with definite, well-thought-out problems, which they wish to solve. They wish to be clinicians and investigators. For instance, we have at present, a man who wishes to spend some years of his life working out questions relating to body temperature. Obviously he has an infinite number of baffling problems facing him in the field. But since he has ideas, he will have the opportunity to work on some of them at least.

We are eager to see our men get results, but research is a good deal like farming. If a man is to produce in medicine, he must sow and cultivate before he can harvest. In other respects, it is like banking. A man may invest his time foolishly in investigation and waste it, or invest it wisely and enjoy real dividends.

Of course, the attitude that demands practical results is not the best. In addition to the great funds back of the Foundation, we have abundant clinical material which is unsurpassed anywhere, and a large group of clinicians interested in the care of the sick of today, as well as the welfare of the sick of the future. Research should not be confined to problems of the next generation, but should endeavor to solve the underlying problems of the present. The latter loom large in the minds of most of us here. How can we solve practical problems that we meet from day to day? The material for clinical investigation is here already, and it is our function to undertake problems as they are presented. Last month a leading clinician from the East stated that in his opinion one of the strongest features of our position in Rochester was the tremendous inspiration and stimulation which we derive daily from our clinical contacts. If we have any of the spirit of investigation, it is impossible to spend the morning seeing cases of unusual interest without being inspired to work out something which will throw light on the unknown, particularly with so large a group of young men who are keen to take up problems, the details of which we may not have time to undertake.

A word or two about some of the investigations in progress at present may not be amiss. In the field of diabetes and carbohydrate metabolism, Dr. Wilder and his associates have been carrying on extremely interesting investigations, which he will probably discuss with you this morning.

In the cardiorenal vascular field, Dr. Keith has been investigating the subject of dehydration, and finds that by employing sugar solution in a little less concentrated form than that employed by Woodyatt, it is possible to dehydrate the blood to the extent of 20, or even 40 per cent, without inducing any great increase in temperature. Dr. Keith, and Dr. Wagener of the ophthalmologic department, have, during the last year, observed from twenty to twenty-five cases of malignant hypertension of a type which is not commonly recognized. The patients had good renal function, by all tests employed, but marked hypertension and definite eye ground changes which are more or less pathognomonic in appearance in some instances. It is important to recognize this group, for many have died within the year, and all from vascular accidents.

Dr. Keith and Dr. Barrier are working on the treatment of edema with calcium. Ammonium chloride is also being used in the same connection. Miss Whelan, Dr. Barrier and Dr. Keith have administered various salts intravenously, and have studied the effects of various inorganic salts on the level of the anions and cations of the blood and urine.

Large doses of sodium rob the body of potassium, and large doses of calcium rob it of sodium.

Dr. Brown and his group have been studying capillaries of nail folds in health and disease. Several hundred cases have been observed, and three or four pathognomonic types of capillaries recognized. Calorimetric studies, by means of a special calorimeter devised by the Department of Physics, are also in progress in relation to the blood flow in the extremities in various vascular diseases.

Dr. Hench has developed the salivary urea index of renal function, which is probably familiar to most of you. He is also making a study of the excretion of mercurochrome, and finds that it is concentrated in the gall bladder.

Dr. Bryan is making a study of hippuric acid synthesis in kidney diseases. His findings confirm those of Kingsbury, and he has also shown that in this synthesis, the liver does not have any direct part in the mobilization of glycocol, although it may have an indirect effect.

In Rochester, we are attempting to develop group investigation analogous to our group practice. In this connection we are carrying on at the present time studies of liver function, investigations in which Doctors Greene, Walters, Snell, Counseller, McVicar and Eusterman are collaborating. In the field of jaundice we have already obtained data of very considerable clinical importance. In the next decade we may witness developments in the field of liver function comparable to those relating to renal function during the last decade. At present investigations are concerned with the value and significance of liver functional tests, rather than with liver function *per se*, a stage which we passed through in the field of renal function studies ten or fifteen years ago.

In closing, I would again lay due emphasis on the incomparable opportunity for clinical investigation offered by this institution. This opportunity, as do all opportunities, carries with it responsibilities. Our laboratories are concerned with the welfare of the sick of today, as well as with those of the future. We must remember that the routine of tomorrow rests on the successful research of today.

Dr. Brown.—An interesting resolution relative to postmortem technic was presented to the Rochester Medical Association at Rochester, New York, by the local undertakers' Association recommending that while doing an autopsy the large vessels be ligated low enough to permit the undertaker to embalm the body satisfactorily. Acting on this recommendation all pathologists in Rochester are carefully tying off all the large vessels before severing from the aorta.

There is one point which was brought up by Doctor Robertson on the sentimental side of relatives of deceased persons that has another side as I see it. A medical student who goes through his four years in a recognized medical school is able to visualize the entire picture of a postmortem examination, and it so happens in his case that in the event of the death of one of his relatives he is able to picture to himself the complete postmortem examination in all details as the request is made for permission. If the examination is made he is really subjected to a double grief and it seems to me it is rather hard on some types of trained medical men to ask them to suffer twofold unless the case warrants it. Now, the layman does not have to go through quite the same experience; he has in his mind no picture of what is to take place and he suffers from the death of the relative alone.

A friend of mine told me of an autopsy carried out in an undertaking room several years ago that shows how a postmortem examination may react on a relative even after he has given his consent to the same. The day was warm and the door of the examining room was left open though unfortunately it was not guarded. The relative paced back and forth on the walk and finally worked around to the back of the building and casually looked in through the door and saw the operator at work on the autopsy that to him was most gruesome. The shriek that came from his lips was that of extreme agony and pain and one that none of us would care to hear. Here of course the whole plan was wrong but it brings to our attention the need of the greatest care in the conduct of our postmortem studies.

I think we have got to be careful and tactful, considering the general conditions and

obtain permission on first, the cases dying of causes unknown, second, on the doubtful or partly obscure cases and lastly on the routine cases with clean cut diagnosis of the causes of death.

It seems to me that tact is most worth while in obtaining postmortem examinations and the impression should be definitely left with the relatives of the deceased whenever it is possible to do so that a favor is being conferred upon them by making the examination and that it is not being made to satisfy the curiosity of a few physicians and students. The body in the postmortem room should be treated with the greatest consideration, first as a mark of respect to the dead who was beloved by some one, sometime, and second, that the embalmer may have a chance to restore the appearance so that no observer can see the lines of incision.

The great purpose of medicine is to prevent and relieve human suffering and it seems to me that the suggested wholesale postmortem examinations would be contrary to the fulfillment of this aim and many needless routine autopsies would be made under this policy that would on completion have added not one item of interest, knowledge or satisfaction to anyone except perhaps the statistician.

The Journal of
Laboratory and Clinical Medicine

EDITORIALS

New Light on Essential Hypertension

NUMEROUS theories have been advanced to explain arterial hypertension. It is well recognized that changes in one or more of the several fundamental factors upon which normal arterial tension depends may give rise to an increase in blood pressure. It was first thought that high blood pressure is always secondary to kidney injury and probably an adjustment to compensate for the increased force necessary to drive the blood through the diseased kidney.

The kidney factor has been perhaps overemphasized. The trend of opinion is towards a chemical explanation for the rise in pressure rather than structural kidney changes. This view is due largely to the separation, as a clinical entity, of a condition in which the arterial hypertension is the primary factor. Janeway gave to the syndrome the name, "chronic hypertensive vascular disease," while Sir Clifford Allbutt designates it, "hyper-

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piesia." "Essential hypertension" is the term most commonly employed. It is agreed that the fundamental change is an abnormal contraction of the arterioles.

The greatest interest in the disease after recognizing the essential anatomic basis has been the search for the pressor substance or substances responsible for the arteriolar spasm. The facts known at present concerning pressor bodies are well summarized by Shaw[1] in his excellent monograph, *Hyperpiesia and Hyperpiesis*. Soluble pressor substances are remarkably few in number. Those occurring in the body are protein in nature and may be of metabolic, bacterial, cellular, or intestinal origin.

Dale and Dixon have described in detail the pressor action of amines such as isoamylamine, p-hydroxyphenylethylamine, and phenylamine which are formed in the course of the putrefaction of protein. These, it is supposed, may be formed in the intestinal tract of man, or possibly by prolonged peptic digestion. Tigerstedt, Bergman and Shaw, have also shown that an extract of the normal kidney cortex, known as renin, will produce a temporary rise in arterial pressure on intravenous injection.

It has not seemed possible to critical observers, however, that any of the pressor substances heretofore described can be responsible for essential hypertension. The pressor action has usually been of a minor degree and evanescent. In most instances the pressor substances have been abnormal products of metabolism. There has been no proof of the presence in the body of the substances which have been demonstrated experimentally to have a pressor action.

Major and Stephenson,[2] of the University of Kansas Medical School, have recently opened up a new avenue of investigation by demonstrating that at least one normal metabolite is a pressor substance to a marked degree. Beginning with the assumption that the pressor substance must be of protein origin, these workers have investigated the properties of some of the better known metabolites. They found that urea, uric acid, creatin, and creatinine are without effect on blood pressure, but that the guanidin bases have remarkable pressor effects. A dose of methyl guanidin varying from 0.1 to 0.2 gm. per kilogram of body weight will double or even triple the blood pressure of a normal dog and maintain this level often for four or five hours. A number of guanidin bases have been studied and all apparently have this pressor effect. Methylguanidin and dimethyl guanidin are normal end products of protein metabolism. Creatinine is a closely related body being methylguanidin acetic acid. Normal individuals excrete about 100 mg. of guanidin bases per day. In experimental uranium nephritis, chronic nephritis with hypertension, and in essential hypertension there is a decrease in excretion as compared with normal individuals.

The guanidin bases have been the subject of numerous investigations especially as a possible cause of tetany. In large doses they produce irregular heart action, a slowing of the pulse, and convulsions. It has been repeatedly emphasized that essential hypertension, uremia and eclampsia have much in common. The fact that the guanidin bases produce the symptoms

characteristic of these allied conditions suggests that it may be the toxic agent common to the three.

This work of Major and Stephenson constitutes probably the most important contribution made to essential hypertension in the past decade. It opens up a new field of study and offers many potential possibilities.

REFERENCES

[1] Shaw, H. Batty: Hyperpiesia and Hyperpiesis, Henry Frowde, London, 1922.
[2] Major, R. H., and Stephenson, W.: Bull. Johns Hopkins Hosp., 1924, xxxiv, 140; 1924, xxxv, 186.

—*R. L. H.*

The Scope and Function of the Laboratory Report

B ROADLY speaking, there are only two methods of practicing medicine: A hasty survey, coupled with a tacit acceptance of the patient's diagnosis of "indigestion" or what not and followed by a more or less effective symptomatic prescription—a method which has been succinctly termed "peddling pills"; or an honest, scientific attempt to diagnose the underlying cause of the symptomatology and to formulate a plan of treatment in accordance with its mechanism.

There is no satisfactory or successful middle course or compromise, even though the latter method may lead through tortuous and devious by-paths before the etiology and mechanism of the associated phenomena comprising the symptoms are laid bare.

The education of the public proceeds slowly—but it proceeds and the proportion of individuals increases who realize the necessity for thorough, careful and systematic procedures directed toward the accumulation of data, the thoughtful consideration of which may lead to the formation of a diagnosis as an essential prerequisite for intelligent treatment and who, therefore, are cognizant of the existence and expect the use of certain methods of greater or less precision in the study of their condition.

Manifestations of disease, in brief, are manifestations of disturbance or loss of function and not infrequently the particular functional imperfection which, because of its interference with the comfort or habits of the patient, has led him to consult the physician is only the end of a devious path leading back to a primary cause only indirectly connected with the presenting complaint and which must be uncovered by, at times, sustained endeavor and laborious search.

Each case, therefore, necessitates a careful survey and no satisfactory or complete survey is possible in these modern days without the introduction and utilization of varied methods of laboratory examination and study, the value of which is directly proportionate to the care and appropriateness with which they are chosen and the skill and keenness with which the results are interpreted.

Unfortunately, however, in many instances the importance and significance assigned to the results of laboratory examinations is predominant and unwarranted and they are too often looked upon as something separate and

apart from all other methods of study and at times even made to replace, supplant, or render apparently unnecessary other and detailed methods of examination.

That this is true cannot be controverted when one considers the number of cases in which the diagnosis of syphilis, tuberculosis, or goiter, for example, rests almost entirely upon the results of complement-fixation tests, sputum examinations, or determinations of the basal metabolic rate; or, what is still more to be decried, such diagnostic possibilities are cast aside because of "negative" laboratory reports.

Because the refinements and elaborations of laboratory technic have, under certain circumstances and with well-defined but not always equally well-appreciated limitations, conferred a definite significance upon positive results, a more or less equal significance has been attributed to negative results without, however, comparable premises to warrant such deductions.

Because laboratory findings of various kinds are a relatively constant feature of certain pathologic entities there has been a tendency to think of such reactions as definitive of the disease in question; to assume that, if their occurrence at times constitutes weighty evidence in favor of the disease, their absence likewise is equally weighty evidence against such a possibility—forgetting that—*always* the occurrence of positive reactions depends, not only on the presence of special stimuli setting in motion the mechanism responsible, but also that the occurrence of the reaction, the detection of "positive" laboratory findings is directly dependent upon and regulated by two important factors: the varying ability of different individuals to respond to similar stimuli, and the perfection, delicacy, and reliability of the methods used to detect and measure such response.

The laboratory report and methods of laboratory examination should not, therefore, be exalted to the neglect and minimizing of other methods of examination and study, which prior to the elaboration of laboratory methods, furnished the foundation upon which the diagnostic superstructure might be erected.

It is not the function of the laboratory *ipso facto* to make the diagnosis or of the laboratory report to furnish the sole or predominant basis for diagnostic assertions. Just as the fluoroscope and the films of the intestinal tract furnish the clinician with a graphic picture of the workings of the tract under examination, so it should be the function of the laboratory report to furnish him with a graphic record from a consideration and interpretation of which he may construct a mental image of the mechanism and functional efficiency of the structures under consideration.

It is not the report, therefore, but its interpretation which is of significance and the value of the laboratory report is hence in direct proportion and indissolubly related to the care and acumen with which the method of laboratory study is planned, and the skill and keenness with which its results are weighed and evaluated *in conjunction with all the information pertaining to the case* elicited by all the methods available and of which the clinician is cognizant.

There are, at present, so many divisions and subdivisions of the laboratory, so many specialized methods of laboratory study, that it will rarely be feasible—especially in private practice—nor is it always necessary or desirable because of the financial outlay demanded of the patient, to subject every case without exception to complete laboratory studies. Procedures of great value and significance in one condition may have relatively little value in another. He who most wisely assorts his laboratory requisitions in accordance with the possibilities to be considered; who chooses them with a definite understanding of the value and significance of the results as applied to the particular case, will utilize the resources of the laboratory most wisely and efficiently and will derive far more of value from its reports than he who makes requisitions in haphazard fashion with the subconscious or tacit hope that, in some mysterious way, the results will whisper the diagnosis and obviate the necessity for other and, perhaps, tedious and time-consuming clinical studies.

In the clinical evaluation of laboratory findings it must be remembered that *it is not the isolated result, the data of single examinations, but the constancy with which abnormal findings occur which*, in the long run, confers the greatest significance

Single urinalyses, agglutination tests, complement-fixation tests, or other determinations are of little value unless interpreted in conjunction with all the other circumstances of the case which may, perhaps, very markedly modify the conclusions to be drawn from them, and unless it is realized that they apply only to the single specimen examined under these circumstances and that they are of far less value and significance than the correlated fact that the apparent abnormalities so detected are constantly found upon subsequent examinations.

The isolated determination of a decreased acidity in a single examination of gastric contents suggests only the *possibility* of hyperchlorhydria, for example, *but the repeated finding renders the possibility a probability to be* correlated with, and perhaps corroborated by, the other available data.

The diagnosis of syphilis may, at times, be practically made by a properly performed, checked, and controlled complement-fixation reaction, but it can never be negatived with an equal degree of assurance by a single negative reaction.

The tendency to associate certain laboratory examinations with certain diseases as predominant, invariable, and pathognomonic evidence of such disease and their absence as equally good evidence of the absence of such a condition, is to be strongly decried as unwarranted by the evidence and tending toward a neglect of clinical examination and study.

The laboratory report should be merely a source of information; a means of supplying the clinician with data not otherwise available, and the data should be supplied and sought for with the distinct understanding that it is to be weighed, evaluated, and interpreted; that it is not, in itself, a complete study or examination, but merely a *phase* of such a study with which all the other information must be correlated.

Its true value, therefore, lies in its interpretation—and the correctness and significance of the interpretation is determined solely by the skill, experience, and acumen of the interpreter.

Upon whose shoulders this responsibility shall rest; whether upon the clinician to whom the report is made, or the clinical pathologist by whom it is constructed, is a matter of debate with much to be said in favor of the utilization of the attainments, experience, and skill of both upon the neutral ground of consultation.

—*R. A. K.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The National Health Series**

IN producing this series the publishers have made a distinct contribution toward the popularization of preventive medicine, paralleling in booklet form the valuable work of "Hygeia" which appears as a periodical. The series of twenty volumes is edited by the National Health Council and each booklet is written by a recognized authority.

"Community Health" is covered by Dr. Armstrong, Executive Officer of the National Health Council, who is well fitted to write upon this subject by virtue of his experience in directing the Framingham Experiment. In "Personal Hygiene" Dr. McLaughlin of the U. S. Public Health Service gives in a direct manner the rules for right living. He describes as clearly as one may of what true health consists. He discusses very simply the application of personal hygiene and emphasizes periodic health examinations. T. Stuart Hart discusses disease of the heart and circulatory apparatus. Lucy H. Gillette in another volume writes upon practical dietetics. In a volume entitled "The Human Machine" Dr. William H. Howell contributes a section on practical physiology.

Jas. A. Tobby, Administrative Secretary of the National Health Council has written a story of "The Quest for Health." Following the high road, he points out the misleading byways, calls attention to the helpful guide posts placed along, and lead us to the end of a pleasant journey. Richard A. Bolt, of the American Child Health Association, writes on babies and Henry L. K. Shaw of Albany Medical College on "The Young Child's Health, his Normal Growth and development, and Problems of Hygiene and Training."

"Cancer, Its Nature, Diagnosis and Cure," is covered by Francis C. Wood, Director of Cancer Research of Columbia University. He follows in general the lines that are emphasized by the American Society for the Con-

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trol of Cancer and incorporates a chapter upon cancer quacks. Dr. C. E. A. Winslow is eminently qualified to discuss communicable diseases.

Each volume is a vest pocket edition with flexible leather covering and may be read in spare moments on the street car, in the train, or while awaiting an appointment. The price is within the reach of all, and the binding is so attractive that the books may well be displayed as a decoration for the library table. All volumes are of approximately equal length. We regret that some are not more detailed, for justice cannot be done to certain of the subjects in the small space allotted. This applies particularly to the contributions on dietetics and on child health.

The medical profession as a whole should become thoroughly acquainted with these volumes and with similar contributions, such as those found in "Hygeia," lest they soon find the wide-awake layman better acquainted with recent developments in the fields of life extension and public and individual health than he is himself.

Protoplasmic Action and Nervous Action*

A TREATISE on the physico-chemical basis of the more general or fundamental properties of living matter. The author approaches the problem from a physico-chemical basis. He feels that the regularity of the living organism presupposes regularity of its component physico-chemical processes. In fact, one of the most striking features of organic processes is their exactitude, which is frequently safeguarded by regulatory devices of the utmost delicacy. The volume contributes a distinct addition to our knowledge of biologic processes, particularly those of irritability and the transmission of stimuli through living matter.

Epitome on Blood Pressure†

A COLLECTION of disjointed statements with reference to blood pressure, most of which are based on existing knowledge. The pages devoted to treatment discuss the value of autocondensation. In fact this appears to be a brochure on autocondensation in the treatment of hypertension. The cost of the book is surprisingly high.

The American Society of Clinical Pathologists

Dr. John A. Kolmer, Philadelphia, Pa.
President

Dr. Frederick E. Sondern, New York
1st Vice President

Dr. W. F. Thomson, Beaumont, Texas
2nd Vice President

Dr. Ward Burdick, Denver, Colorado
Sec'y-Treas.

The A. S. C. P.

The A. S. C. P. in its brief period of existence has already rendered great service to the cause of scientific medicine by helping to elevate the status of the clinical pathologist in his relation to the clinician and to stimulate the use of laboratory aids in diagnosis.

Older and long established societies may be quiescent during a whole year and only show signs of activity at the annual meeting. The American Society of Clinical Pathologists, however, is a young, vigorous and actively growing organization with strong proliferative tendencies aiming to keep the members constantly in touch with one another and spurring them on to better scientific and technical perfection of their specialty.

MEMBERSHIP IN THE A. S. C. P.

The secretary of the A. S. C. P. reports a flood of inquiries regarding membership in this national body. The clinical pathologists throughout the country are recognizing the benefit of organization and appreciate the excellent results achieved by the A. S. C. P.

Application blanks and information may be obtained from Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.

CLINICAL AND EXPERIMENTAL

AN ADEQUATE LABORATORY SERVICE IN THE MODERN HOSPITAL*

By WARD BURDICK,† M.D., DENVER, COLO.

AS the representative of the American Society of Clinical Pathologists, it becomes my privilege to convey the appreciation of your courtesy in extending the privilege of appearing before this convention for the purpose of expressing views on matters which so mutually concern us.

Upon receipt of the invitation to address this meeting on the subject of "An Adequate Laboratory Service in the Modern Hospital," a communication was directed to the Fellows of the American Society of Clinical Pathologists, enclosing a questionnaire covering this subject, and this paper is a digest of the answers received. It has been the endeavor to make it express the net result of the experiences of all of the Fellows of the Society which I represent.

The first question for discussion, it would seem, should be:

PERSONNEL

On this matter, there is a striking unanimity of opinion among the clinical pathologists. With reference to the head of the organization, O. J. West, Seattle, Washington, says: "All hospital laboratories should be in charge of a clinical pathologist on whom the responsibility for all work should rest." These words express the sentiment of all clinical pathologists from whom replies were received. This brings us to the pertinent question, "What is a clinical pathologist?" At the last Annual Convention of the American Society of Clinical Pathologists, the following definition was adopted:

"A clinical pathologist is a physician who has specialized in clinical microscopy, chemistry, serology, bacteriology, pathologic histology, and gross morbid anatomy, for a period of two years under the direction of a clinical pathologist as herein defined."

Thus, at the outset, we find ourselves at variance with the American College of Surgeons on a very important point. Referring to the tenth Year Book of the College, issued during the present year, on page fifty-three, paragraph v, under the heading of "The Minimum Standard of Hospitals," reads as follows: "That clinical laboratory facilities be available for the study, diagnosis and treatment of patients, these facilities to include at least chemical, bacteriologic, serologic and histologic service in charge of trained technicians." John Hewat, Director of Laboratories, Department of Health, State of Maine, states: "If a hospital has a fully equipped laboratory capable of undertaking all laboratory procedures, the object of the laboratory is defeated, unless the responsible head is qualified, as defined by the American Society of Clinical Pathologists. The technical part of the work can, perhaps, be left to trained technicians, but a knowledge of the fundamentals of medicine is very essential in the interpretation of the results; therefore, the head of the laboratory should have a Medical Degree." "For," says James C. Todd, Professor of Clinical Pathology, University of Colorado, "it would seem reasonable to ask that all clinical laboratory work of a hospital be done under the direct control of a clinical pathologist, and that there be a definite department of clinical pathology recognized at staff meetings."

"In small hospitals," according to E. H. Buttles, Mary Fletcher Hospital, Burlington, Vermont, "perhaps under one hundred beds, he need not be a full-time man, but should have definite hospital hours." Harry Corper, National Jewish Hospital for Consumptives, Denver, insists that, "Every hospital of one hundred bed capacity should have a full-time resident clinical pathologist, with an appropriate staff of assistants." To these sentiments, every Fellow of the American Society of Clinical Pathologists subscribes, and we venture to say that were the American College of Surgeons to rewrite Paragraph v in the light of present day advancement in our field, the term "clinical pathologist" would supplant that of "technician."

That there is a place under the sun for the technician is the opinion of all clinical pathologists, for says Michael G. Wohl, Methodist Hospital, Omaha, "Lay technicians are valuable and absolutely necessary, yet when it comes to interpreting laboratory findings as clinical symptoms, a Degree in Medicine and several years' experience in hospital work are absolutely necessary"; but, J. H. Black, of Dallas, Texas, warns us that, "they should not be given autonomous positions." "Technicians," says A. H. Sanford, Mayo Clinic, Rochester, Minnesota, "are, in my experience highly trained laboratory workers, preferably women working under someone's direction." My personal opinion with reference to this matter is, it must be admitted, somewhat at variance with that of the majority of my colleagues, as it has long been the belief of the writer that the chief assistant in the hospital laboratory should be a potential clinical pathologist, a young man or woman who, having graduated in medicine and having served a proper internship in a general

hospital, elects to enter the field of clinical pathology as a life work. How else, it may be asked, may clinical pathology be perpetuated? The ever-widening circle of the application of laboratory methods to diagnosis, demands skilled physicians, especially trained to meet the demands of the modern hospital, but from whence are the clinical pathologists of the future to come? I do not know of a single young physician serving an apprenticeship in a laboratory. Instances may exist, and probably do, but they are hopelessly few. There are but two answers to this question, first, the encouragement of young physicians of scientific inclination to enter the laboratory as first assistants, and, second, to again quote James C. Todd, Professor of Clinical Pathology, University of Colorado, "See to it that there is a strong department of clinical pathology in every medical college."

Returning, then, to the subject, it may be said that the personnel of the hospital laboratory should include first, a clinical pathologist; second, to interpose a personal view, a young physician in course of training to become a clinical pathologist; and, third, as many technicians as the requirements of the institution may demand

LOCATION, SIZE AND ARRANGEMENT, OF LABORATORY SPACE

That the laboratory should be on the uppermost floor, remote from the dust, noise and vibration of the street, with a northern exposure and located as near to the operating room as possible, is the consensus of opinion expressed by the clinical pathologists; Charles Sietz, Evansville, Indiana, says, "Its location should be such that it will be freely accessible to the members of the hospital staff, in order to invite and encourage consultation with the clinical pathologist. In the event of its not being convenient to place it near the operating room, a small laboratory for frozen sections of fresh surgical material should be provided adjoining the surgical department." There should be at least five hundred square feet for every one hundred beds, "and the arrangement," says John Kolmer, University of Pennsylvania, with whom most clinical pathologists agree, "should depend upon the service required; but for a one hundred and fifty bed hospital, I believe that the laboratory should be divided into the following departments with space set aside for each; (a) For the preparation of tissues and culture media, and general sterilization; (b) Clinical pathology and clinical bacteriology; (c) Serology, with special reference to the Wassermann test; (d) Biochemistry." To this allotment, many of our Fellows would add a private office and record room for the clinical pathologist in charge, and it is generally preferred that the above departments be provided with separate rooms. "In any event," says A. H. Sanford, Mayo Clinic, Rochester, Minnesota, "don't shunt the laboratory off into the basement."

"As to equipment," laconically replies one clinical pathologist, "the best is none too good"; but, W. W. Hall, Watertown, New York, advises, "the best of apparatus should be obtained; do not buy apparatus until there is a demand for it; discard apparatus as soon as defective, and always buy duplicate apparatus that is breakable. In the opinion of practically all of the clinical pathologists, however, the fundamentals of equipment are as

follows: Autoclave, Arnoll Sterilizer, Centrifuge, Incubator, Microscope, Refrigerator, Microtome, Basal Metabolometer, Colorimeter, Water-Still, Hemocytometers, and appropriate glass-ware, instruments, and chemicals for carrying on clinical microscopy, chemistry, serology, bacteriology, and pathologic histology, which, thinks A. V. St. George, Bellevue Hospital, New York City, "can usually be purchased for around $1,500.00. This would equip the average one hundred to one hundred fifty bed hospital laboratory. It is obvious, however, that the size of the institution will largely govern the amount of apparatus required." The writer would be inclined to make the latitude "around $1,500.00" rather liberal so that it might be stretched to $2,000.00 if need be, particularly in view of present prices.

<div align="center">SCOPE OF SERVICE</div>

Now, in terms of golf, the ball has been knocked out into the rough, for here will be encountered several moot questions: first, what tests, if any, should be done as a matter of routine, taking into account both medical and surgical cases? "Routine blood and urine analysis," answers George L. Bond, Grand Rapids, Michigan, with whom ninety-six per cent of the clinical pathologists agree. "These tests reveal so many conditions that influence treatment, that the attending physician should have them in every case"; L. H. Cornwall, New York City Hospital, adds, "They are a necessary part of every record before diagnosis can be made with accuracy." Foy C. Payne, Dayton, Ohio, who voices the opinion of 47 per cent of his colleagues, states, "A Wassermann should be done routinely on all chronic cases." We are also reminded by several not to overlook a routine throat culture and vaginal smear in children, as well as the determination of coagulation and bleeding time on all tonsil and adenoid cases. Other tests, such as basal metabolism, blood chemistry, kidney function, blood typing, etc., were occasionally mentioned in the replies; but, A. B. Giordano, South Bend Medical Laboratory, South Bend, Indiana, says, "These three tests, namely, complete blood count, urine analysis and Wassermann reaction, will in a great many cases reveal evidence of disease process, of which neither the patient nor physician is aware." My own views as to hospital laboratory routine coincide with those expressed by John R. Porter, Rockford, Illinois, who says, "Urines and blood counts on all, sputum on all chest cases, blood culture on all temperatures of one hundred and two degrees Fahrenheit; throat cultures on all suspects and undiagnosed cases; stools on all diarrheas, and smears and cultures on all discharges; for the average clinician does not know exactly what laboratory examinations his patients should have, so he does not order them, and the above will many times clear up the case before the tests suggest themselves to him." "Furthermore," as Father Mouliner is wont to remind us, "the patient in the hospital is the object around which everything should revolve." The clinical pathologist should be one of the patient's closest relatives during his stay in the institution, and should not be hampered by formalities during the application of his endeavors to elucidate the cause of disease. His advances should be welcomed by attending physicians as an integral part of hospital service to which his patient is entitled.

With reference to surgical cases, it should be an axiom that no cutting operation, however small, should be undertaken—with the ever-present liability of infection—without first ascertaining the status of the blood as a matter of record; and, says Dr. Edward Mugrage, Assistant Professor of Clinical Pathology, University of Colorado, "The urine should always be checked in order to observe any renal disturbances which might otherwise be overlooked, and which would influence the choice of the anesthetic." It is the consensus of opinion that the histologic structure of all tissues removed at operation should be made a matter of record, not only as a check on clinical diagnosis, but as evidence which may be of inestimable value to the patient or his relatives at some future period, to say nothing of the statistical worth of such archives.

Another question: Should the activities of the hospital laboratory be limited to the institution alone, or may its scope of service reach out beyond the confines of the hospital? On this point, the clinical pathologists seem to be equally divided. "Most emphatically, yes!" says B. W. Rhamey, Fort Wayne, Indiana, representing one extreme, "They should confine their activities to the institution alone. Hospitals have no business going into competition with men making a specialty and life work of this branch of medicine. That would be another phase in the advance toward State Medicine"; but Ralph G. Stillman, Department of Pathology, New York Hospital, representing the other extreme, states, "I see no reason why the hospital laboratory should confine its activities to the institution, providing its personnel is large enough to handle the necessary work. The offer of diagnostic aid to practicing physicians in the neighborhood of the hospital, and on the hospital staff, broadens the service of the institution to the community, and establishes for the institution a permanently interested clientele that is of value both to the institution and to the community. No hospital that fails to act as a center for the dissemination of information concerning the prevention, diagnosis and treatment of disease is fulfilling its entire duty." I prefer the middle ground with M. W. Lyon, Jr., of South Bend, Indiana, who says that "if the clinical pathologist in the hospital is the only one in the community, he may reach outside; but, if there are private practicing clinical pathologists in the community, the hospital laboratory should keep to itself." For, after all, the development of clinical pathology as a specialty in medicine should not be impeded.

FINANCING

We are now face to face with another problem reflecting upon the scope of service of the hospital laboratory, namely, that of financing this department of the institution. This brings us deeper into the thicket of uncertainty, and constitutes the "fly in the ointment" of cooperation in this phase of hospital standardization.

Adequate laboratory service will not be realized until the true status of the clinical pathologist is recognized by all classes of physicians. From the most remote time up to about twenty years ago, the contact of the medical profession with the laity was chiefly through one man—the family doctor.

About this time, there appeared a third party, the clinical pathologist, whose function it was to develop methods for the practical application of scientific facts which were rapidly being brought to light by the medical research laboratories of the world. The newcomer was acclaimed by physicians and surgeons alike, who paid their compliments with a shower of material to examine gratis. But it was soon evident that a definite department of medicine was developing which required to be maintained and the present confusion proceeds from the reluctance on the part of the firmly grounded practitioner to so modify his demands upon the public that the clinical pathologist might derive his share of financial support. A comparison of the skill of the clinical pathologist involved in condemning a breast or a limb, with that of the surgeon who removes it, would be as likely to balance in favor of the former as the latter; but a considerable difference in compensation still obtains. That the public pays enough for medical service is obvious, and it is hoped that the handwriting on the wall will not escape the attention of those concerned, for the person who is not indifferent to the signs of the times may with safety predict that State Medicine will be the inevitable outcome if the cost of medical, and particularly surgical, service continues to increase.

As to methods in vogue at the present time for financing clinical pathology in the hospitals, an analysis of the questionnaires reveals a complete lack of uniformity, and one is at a loss to make any recommendations. For the present, however, we must proceed upon the thesis that the clinical pathologist is a consultant in medicine and should be compensated accordingly the same as the surgeon or internist. Regarding the so-called "flat fee," a majority of the clinical pathologists agree with Dr. Philip Hillkowitz of Denver, who says, "that a flat fee is permissible only for routine urine and blood examinations; that the hospital laboratory should be financed by charging each patient for work performed; while, for the more complicated tests, the usual fees in vogue should be charged and after proper deduction is made from the total monthly laboratory income for expense of laboratory supplies, salary of technicians, interest on investment, expense of rooms, light, heat, etc., the balance should be regarded as the compensation of the clinical pathologist."

In closing, may I suggest the appointment of a committee by the American College of Surgeons to cooperate with a similar committee from the American Society of Clinical Pathologists, to formulate a plan of financing the department of clinical pathology in the hospital?

To summarize, then, the personnel of the hospital laboratory should consist of a clinical pathologist in charge, a young physician in training for clinical pathology, and as many technicians as the work of the institution may require. It should be located adjacent to the operating room, preferably on the top floor, with a northern exposure, and subdivided into five rooms, including a private office for the clinical pathologist. The equipment should be adequate for the carrying on of all the necessary tests in clinical microscopy, pathologic histology, bacteriology, serology, and chemistry. As a matter of routine, the laboratory department should determine the condition of the

blood and urine of all patients entering the hospital, and write into the records of the institution the results of histologic examination of all tissues removed at operation. A Wassermann test should also be done as a matter of routine, at least upon chronic cases. The clinical pathologist should be freely employed as a consultant by the staff, and it should be considered his privilege and duty to perform any tests which, in his judgment might throw light on the condition of any patient. The activities of the hospital laboratory should be confined to the institution alone, unless it happens to be the only available center of clinical pathology in the community. The evolution of a satisfactory method of financing clinical pathology in the modern hospital is a matter for future determination and especially in the province of the committee suggested.

THE INFLUENCE OF THE NATURAL ANTISHEEP HEMOLYSINS OF HUMAN SERA UPON THE PRODUCTION OF ANOMALOUS REACTIONS IN THE FIRST TUBE OF KOLMER'S QUANTITATIVE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By Robert A. Kilduffe, A.B., A.M., M.D., Los Angeles, Calif.

IN his original communication detailing the technic of a new quantitative complement-fixation test for syphilis in which the quantitative factor is primarily dependent upon the testing of five graduated amounts of serum— 0.1, 0.05, 0.025, 0.005, and 0.0025 c.c.—Kolmer[1] called attention to the fact that, occasionally, a positive serum will show less fixation in the first tube of the series carrying 0.1 c.c. of serum than in the second and subsequent tubes carrying 0.05 c.c. or less, thus producing such a reaction as, for example, 2 4 4 4 0.

This observation has been corroborated by all who have worked with the method and, in my own experience, occasional sera have even exhibited complete hemolysis in the first tube of the series when hemolysis has been inhibited to a greater or less degree in the subsequent tubes, thus producing such a reaction as 0 3 3 0 0 or 0 4 3 0 0.

The natural assumption is to ascribe this type of reaction to the presence of natural antisheep hemolysins in the serum tested in amounts sufficient in 0.1 c.c. to cause marked or even complete hemolysis of 0.5 c.c. of 2 per cent sheep-cell suspension, and to assume that the dilution of the serum in the subsequent tubes of the series so reduces the hemolysin content that its effect is masked or negligible.

The practical importance of such reactions lies in the fact that their occurrence furnishes a potential source of error in methods using only a single serum dose of 0.1 c.c.

The assumption that these reactions are entirely due to the presence of natural antisheep hemolysins is logical and, apparently, reinforced by the fact that such reactions have not as yet been encountered with spinal fluids in which natural hemolysins are not found. It is opposed, however, by the fact that in the same communication Kolmer notes their occurrence when using an antihuman hemolytic system and also with hemolysin-free sera, as a result of which he believes them to be due, possibly, to the presence of other and as yet unknown serum constituents which may, in some way yet to be elucidated, interfere with the interaction of complement, antigen, and reagin.

A similar view is held by Detweiler[2] who calls attention to "some peculiar substance present in some sera which causes hemolysis in exactly the same fashion (as hemolysin) but which is not removed on exposure to sheep cells."

The same observer cites Zinsser[3] as also having noted the same phenomenon.

The occurrence of these anomalous reactions as brought out by Kolmer's quantitative method, while relatively infrequent, has a very distinct, important, and practical bearing on complement-fixation technic in general, for, if the reaction is in any way related to the hemolysin content of the serum tested, it becomes an important source of possible error in methods using only a single serum dose of 0.1 c.c.; and, even if it is not related to the presence of natural hemolysins, it still remains as a potential source of false negative reactions.

In the quantitative method described by Kolmer these anomalous reactions are of little practical importance as affecting the reading of the test, for the degree of positiveness is determined, primarily, not by the degree of fixation obtained in any one serum dose, but by the smallest quantity of serum which gives fixation of any degree. Thus, a 0 4 4 4 0 and 2 4 4 3 0 reaction would both be classed as very strongly positive because both show fixation in the fourth tube or in 0.005 c.c. of serum.

Because, however, such a reaction as 0 4 4 4 0 is a possible source of confusion to those accustomed to readings obtained with a single serum dose, and because of the inherent interest of the problem, it seemed of interest to study the problem and the present communication is the result of such a study.

In classing these reactions as a possible source of confusion it is, of course, plain that this is not as apt to be true of the serologist as of the clinician to whom the report is sent; but as long as the clinician is to assume, without exception, the sole prerogative of the interpretation of the complement-fixation reaction, and as long as too frequently, unfortunately, decisions relative to diagnosis and prognosis are to be based upon the results of isolated serologic examinations, it is necessary insofar as is possible, for the laboratory to bend every effort toward clarifying the methods of making and reading the results of the test.

The plan of this investigation was influenced by the following premises:

If the anomalous reactions in question are due entirely, or even in large measure, to the influence of natural hemolysins, then the incidence of both

should be comparable. Strict parallelism need not be looked for necessarily because of the influence of varying amounts of syphilitic reagin in the sera with resultant more or less complete and relatively stable fixation of complement for, while it has been shown that, within certain limits, a deficiency of complement may be compensated for by an excess of hemolysin and the reaction of a weakly positive serum thereby masked or even lost entirely, the influence of an excess of hemolysin upon the reaction obtained with a strongly positive serum containing large amounts of reagin is, to all intents and purposes, negligible, *especially when the serum is tested in amounts of less than 0.1 c.c.*

The plan adopted, then, was:

1. To determine the incidence of natural antisheep hemolysins in all the sera examined.

2. To determine the incidence of all sera giving anomalous reactions in the first tube.

3. To compare the incidence of 1 and 2.

4. To determine the incidence in the sera giving anomalous reactions of antisheep hemolysin in amounts sufficient in 0.1 c.c. to completely or even partially hemolyze 0.5 c.c. of 2 per cent sheep-cell suspension; to absorb the hemolysins from such sera, and retest them and thus determine the effect of absorption of the natural antisheep hemolysins on the reaction in the first tube of the quantitative test.

I. The Incidence of Natural Antisheep Hemolysins in Human Sera.

To determine this, to 0 1 c c. of inactivated serum (15 minutes at 56° C.), were added 1 c.c. of 1:30 complement carrying two "full" units as determined by Kolmer's method of complement titration, and 0.5 c.c. of 2 per cent sheep-cell suspension, the tubes then being incubated for one hour in the water-bath at 38° C. Readings were then made for the presence and relative amount of natural antisheep amboceptor.

In all, a total of 2,180 sera were thus examined with the following results:

Sera containing sufficient hemolysin in 0.1 c.c. to cause complete hemolysis: 1,292 or approximately 50 per cent.

Sera containing sufficient hemolysin to cause marked but not complete hemolysis: 70 or approximately 3 per cent.

Sera containing only sufficient hemolysin to give a trace of hemolysis: 26 or approximately 1 per cent.

Sera without natural hemolysin: 729 or approximately 35 per cent.

The total incidence of hemolysin-containing sera was, therefore, 1,380 or approximately 60 per cent.

II. The Incidence of Anomalous Reactions in the First Tube of the Quantitative Test.

Of the 2,180 sera subjected to the quantitative complement-fixation test according to the method of Kolmer, anomalous reactions occurred in the first tube in 37 sera or approximately 1 per cent.

They may be divided into two groups: *one group of eleven sera gave reactions characterized by complete hemolysis in the first tube with varying*

degrees of fixation in the subsequent tubes of the series; and a second group, comprising twenty-six sera in which there was fixation in the first tube but always of lesser degree than that obtained in the second tube carrying 0.05 c.c. of serum.

It is highly important to note that, in the group of eleven sera giving complete hemolysis in the first tube, there were two sera which did not contain any natural hemolysin.

III. A comparison of the incidence of the natural antisheep hemolysin and of the incidence of anomalous reactions at once indicates that the presence of the former, even in large amount, cannot be the sole factor concerned in the production of the latter; for, while hemolysins were present in large amount in 1,292 sera, anomalous reactions occurred in only 37 sera.

Moreover, of the eleven sera giving complete hemolysis in the first tube, there were two which did not contain natural antisheep amboceptor and in which the reaction, therefore, cannot be ascribed to its presence and must be ascribed to unknown serum constituents other than hemolysin.

The results of the investigation thus far, therefore, suffice to clearly demonstrate the fact that anomalous reactions of the character in question may and do occur in the absence of natural antisheep hemolysin, and that the presence of natural hemolysin is not necessarily the cause of such reactions.

The relation of the natural amboceptor to the production of such reactions will be touched upon in the subsequent phases of the investigation detailed below.

IV. The incidence, in sera giving anomalous reactions, of antisheep hemolysins in amounts sufficient in 0.1 c.c. to completely hemolyze 0.5 c.c. of 2 per cent sheep-cell suspension, and the effect upon the anomalous reaction of the absorption of the natural amboceptor from the sera.

Of the 37 sera in question 34 contained sufficient hemolysin to completely hemolyze the test dose of cells. These were treated with sheep cells to absorb the amboceptor and retested for the degree of complement fixation after absorption.

At the outset of this portion of the study some little difficulty was encountered in finding a method satisfactory for the complete absorption of hemolysins without the coincident development of thermostabile anticomplementary properties in the serum after absorption.

A method was desired which would be rapid, simple, and efficacious and that of Kahn[4] was finally chosen. This method consists simply of the addition of washed and packed sheep cells to the serum in the proportion of one drop of cells to one centimeter of serum and the subsequent removal of the serum by centrifugation after standing for ten minutes at room temperature.

The cells were added to the serum from a 1 c.c. pipette graduated in $\frac{1}{100}$ c.c., both the serum and the cell quantities being approximated and not accurately measured.

Kahn and Lyon[5] have shown that this procedure is capable of absorbing

1,800 units of amboceptor from 1 c.c. of serum after ten minutes at room temperature, so that the method is efficacious as well as rapid and simple.

After absorption the sera were removed by centrifugation and stored in the ice chest overnight. The following morning the sera were again inactivated for 15 minutes at 56° C. and again tested by the quantitative method.

At the same time that the second test was made, to 0.1 c.c. of serum were added 1 c.c. of 1:30 complement carrying two "full" units, and 0.05 c.c. of 2 per cent sheep-cell suspension, the tubes then being incubated for one hour in the water-bath at 38° C., when a reading was made to determine if the amboceptor had been completely absorbed.

Absorption was found to have been complete in every instance and in no instance did the development of thermostabile anticomplementary substances occur.

The results of the complement-fixation tests before and after absorption are shown in the tables.

Over and above the question at issue, a study of the tables brings out several points of interest.

In Table I, while the sera in which natural hemolysins were absent demonstrate conclusively the fact that negative reactions may occur in the first tube through the action of unknown serum constituents, the remainder of the series evidences, by the results of the test after absorption, that the presence of natural antisheep hemolysins may be a factor of importance in the causation of this type of anomalous reaction, as fixation in varying degree was obtained in the first tube after absorption in every instance.

Table II brings out two facts of interest and importance:

a) While the strength of the reaction was definitely increased in the first tube as a result of absorption of hemolysin in 14 sera, in 12 sera no increase in the strength of the reaction occurred after absorption, so that, in approximately 50 per cent of these sera the anomalous reactions occurred independently of the presence of natural hemolysin, and these reactions, had the sera simply been tested for hemolysin content without absorption and retesting, would have been without hesitation attributed entirely to the hemolysin content.

This, however, was not the case and the mechanism of their occurrence remains to be explained.

b) In a perceptible number of the retested sera the strength of the reaction was slightly decreased as a result of absorption and reinactivation; for example, where fixation was obtained in the fourth tube before absorption, on retesting fixation did not extend beyond the third tube.

The explanation of this, in all probability, lies in the possible destruction of small amounts of reagin during the second inactivation period. In view of the fact, however, that the total period of exposure to 56° C. was not longer than that usually made use of heretofore, this explanation may not be the true one and other factors may be at work. It is possible, also, that the effect of exposure to heat may be more clearly brought out in the higher dilutions of the serum.

TABLE I

EFFECT OF ABSORPTION OF HEMOLYSIN ON SERA GIVING COMPLETE HEMOLYSIS IN THE
FIRST TUBE

NO.	BEFORE ABSORPTION	AFTER ABSORPTION
1.	0 1 1 0 0	2 1 0 0 0
2.	0 1 4 4 2	4 4 4 0 0
3.	0 4 4 4 0	4 4 4 0 0
4.	0 4 4 4 0	2 2 2 0 0
5.	0 4 4 4 0	4 4 4 4 1
6.	0 4 4 0 0*	0 4 4 0 0
7.	0 2 4 1 0	4 4 4 0 0
8.	0 2 2 2 0	4 4 4 0 0
9.	0 1 1 1 0	4 4 4 4 0
10.	0 4 4 0 0	4 4 4 0 0
11.	0 4 4 0 0*	0 4 4 0 0

*Serum does not contain natural antisheep hemolysin.

TABLE II

EFFECT OF ABSORPTION OF HEMOLYSIN ON SERA GIVING LESS FIXATION IN TUBE 1 THAN IN
TUBE 2

NO.	BEFORE ABSORPTION	AFTER ABSORPTION
1.	2 4 4 0 0	2 4 4 0 0
2.	1 3 3 1 1	4 4 0 0 0
3.	1 4 4 0 0	1 4 4 0 0
4.	3 4 4 0 0	4 4 4 0 0
5.	3 4 4 0 0	3 4 4 0 0
6.	2 1 1 1 0	4 4 4 0 0
7.	1 4 4 0 0	1 4 4 0 0
8.	1 4 4 0 0*	1 4 4 0 0
9.	3 4 3 2 0	4 4 4 0 0
10.	1 4 4 0 0	4 0 0 0 0
11.	1 4 4 0 0	4 4 4 0 0
12.	2 4 0 0 0	4 2 0 0 0
13.	1 4 0 0 0	4 1 0 0 0
14.	1 4 0 0 0	1 4 0 0 0
15.	2 3 2 2 2	2 3 3 3 0
16.	1 2 2 2 0	4 4 4 1 0
17.	1 2 0 0 0	4 0 0 0 0
18.	1 4 4 0 0	4 4 4 0 0
19.	3 4 0 0 0	4 4 4 0 0
20.	1 2 0 0 0	1 2 0 0 0
21.	3 4 0 0 0	3 4 0 0 0
22.	3 4 0 0 0	3 4 0 0 0
23.	1 2 1 0 0	4 4 4 0 0
24.	1 2 0 0 0	1 2 0 0 0
25.	1 2 0 0 0	4 4 0 0 0
26.	1 2 0 0 0	4 4 0 0 0

*Serum does not contain natural antisheep hemolysin.

This finding is purely of interest only in connection with the interpretation of the reaction as there will be but little difference in the clinical significance of a four-tube or a five-tube fixation—both will be very strongly positive and the patient in dire need of systematic treatment.

CONCLUSIONS

1. In Kolmer's quantitative complement-fixation test for syphilis a positive serum may give a negative reaction in a dose of 0.1 c.c. and a positive reaction of varying degree in doses of 0.05 c.c. of serum or less.

2. Such anomalous reactions may occur in the absence of natural hemolysin in the serum tested and are, therefore, occasionally due to the presence of serum constituents whose nature and mode of action are, as yet, unknown.

3. In a definite proportion of anomalously-reacting sera the reaction is due to the presence of relatively large amounts of natural antisheep hemolysin.

4. Anomalous reactions in the first tube of Kolmer's quantitative test may occur after the absorption of the natural antisheep amboceptor, and absorption of hemolysin from hemolysin-containing sera may be without effect upon the anomalous reaction.

5. In a definite proportion of hemolysin-containing and anomalously-reacting sera, while the strength of the reaction in the first tube may be increased in the first tube by the absorption of the natural antisheep hemolysin, the ultimate dilution in which the serum reacts after absorption may be affected and decreased.

6. In Kolmer's quantitative method these anomalous reactions are of no practical importance and do not necessitate prior absorption of the sera, as the strength of the reaction is not materially affected by the anomalous reactions occurring in the first tube.

7. Unknown serum constituents, not removable by exposure to sheep cells and capable of causing hemolysis, are a potential, though infrequent, source of error in the production of false negative reactions *when only a single dose of 0.1 c.c. of serum is tested* in complement-fixation methods for syphilis.

REFERENCES

[1]Kolmer, J. A.: A New Complement-Fixation Test for Syphilis Based Upon the Results of Studies in the Standardization of Technic, Am. Jour. Syph., January, 1922, vi, 1.

[2]Detweiler, H. K.: Practice of Medicine, Tice: Infection and Immunity, Ed. 1, 1923: W. F. Prior Co., Hagerstown, Md., page 295.

[3]Zinsser: Note 2, personal communication.

[4]Kahn, R. L.: A Simple Method for the Removal of Natural Antisheep Amboceptor from Human Sera, JOUR. LAB. AND CLIN. MED., January, 1921, vi, 4.

[5]Kahn, R. L., and Lyons, D. S.: The Affinity of Sheep Corpuscles for Antisheep Hemolysin, Jour. Infect. Dis., December, 1921, xxix, 651.

MEASUREMENTS OF URIC ACID IN BLOOD BY VARIOUS METHODS*

By Wm. G. Lennox, M.D., and Marie F. O'Connor, B.S., Boston, Mass.

IN the eleven years which have elapsed since Folin and Denis[1] published their colorimetric method for the measurement of uric acid in blood, numerous improvements in the method have been made. These modifications have resulted in a greatly simplified technic, but the values obtained have

varied, the tendency being toward higher figures. By the original method 2 mg. of uric acid per 100 c.c. of blood was considered the upper limit of normal, whereas by the Folin-Wu[2] method, published in 1919, the upper limit is 3.0 or 3.5 mg.

Benedict[3] in 1922 proposed a further simplified direct method which does away with the preliminary precipitation of uric acid. Folin[4] has accepted this modification. He believes[5] that "there is scarcely place for further improvements" in the method of uric acid analysis. Benedict[3] found that his new procedure gave higher values than the Folin-Wu method. The increase of the new over the old method in 40 per cent of the determinations was from 0.6 to 2.0 mg. per 100 c.c. of blood. Discrepancies between the two methods were greatest in bloods containing low concentrations of uric acid. Brown and Raiziss[6] report somewhat similar results.

Folin[4] says that his present direct method (1922) gives values only 0.1 to 0.2 mg. higher than the Folin-Wu method. Using the new Folin procedure without modification (except that we did not find it necessary to use lithium oxalate as an anticoagulant in order to secure clear filtrates) we have measured the uric acid in the blood of a large group of patients with epilepsy.[7] We obtained values much in excess of the limits of normal as stated by Folin for this method. Table I summarizes our findings. Of the 114 determinations on blood drawn from two to five hours after the subjects had eaten, all but four gave values between 2.5 and 4.6 mg. per 100 c.c. of whole blood. Of 79 determinations made on blood drawn from patients (otherwise unselected) who had not eaten for twelve to fifteen hours, thirteen were above 4.6 mg. The average of the readings for this group was 0.5 mg. higher than the average for the nonfasting group. We have shown elsewhere[8] that prolonged starvation uniformly results in a greatly increased concentration of uric acid in the blood. It is possible that occasional small increases might occur in persons who had been without food for as short an interval as 12 hours.

None of the subjects recorded in Table I had abnormal concentrations of nonprotein or urea nitrogen in the blood, or showed other evidence of nephritis. Intensive study of a number of them revealed no evidence of faulty uric acid metabolism. As a further check on our results we measured the uric acid in the blood of 20 known normal persons, mostly students. Blood from these was obtained in the morning before breakfast. Concentrations of blood nonprotein and urea nitrogen were normal. Concentrations of uric acid varied from 3.3 to 5.7 mg., with an average of 4.5 mg. per 100 c.c. of blood. It is possible that these results were somewhat high because blood was taken under fasting conditions. The number of these determinations is too small to permit any statement concerning the normal blood uric acid limits. However, it seems probable that if Folin's new direct method is adopted, the upper limit of uric acid in the blood of normal, nonfasting individuals will need to be raised from the present level of 3.0 or 3.5 mg. to a new level of 4.5 or 5.0 mg. per 100 c.c. of whole blood.

These unexpectedly high uric acid figures led us to make parallel deter-

minations by other methods. Blood for these analyses was taken from normal and epileptic subjects before and during starvation. Table II shows the relationship in 27 instances between uric acid concentrations as measured by the Folin-Wu (1919) and the Folin (1922) methods. In all but three determinations, the values by the new direct procedure exceed those by the old method by more than 0.5 mg. Discrepancies between the two methods hold for both normal and abnormal concentrations of uric acid. There is no constancy in the amounts of difference.

Table III gives a comparison of results by the new method of Benedict and of Folin. The differences are small. Of the 30 determinations the Folin method is higher by more than 0.5 mg. in five. The average of all determinations by the Folin method is 0.2 mg. higher than by the Benedict.

TABLE I

DATA CONCERNING 193 MEASUREMENTS OF URIC ACID IN THE BLOOD OF PATIENTS WITH EPILEPSY

BLOOD TAKEN NO. OF HOURS AFTER FOOD	NO. OF SUB- JECTS	NO. OF MEASURE- MENTS	MG. PER 100 C.C. WHOLE BLOOD			PER CENT ABOVE 4.6 MG.
			MAXIMUM	MINIMUM	AVERAGE	
2-5	79.	114	6 0	2.5	3.5	4.
12-15	69.	79.	6 5	2.3	4.0	16.

TABLE II

CONCENTRATION OF URIC ACID IN BLOOD BY THE METHODS OF FOLIN (1922) AND FOLIN-WU (1919)

MG. PER 100 C.C. WHOLE BLOOD					
FOLIN (1922)	FOLIN-WU (1919)	EXCESS OF FOLIN OVER FOLIN-WU	FOLIN (1922)	FOLIN-WU (1919)	EXCESS OF FOLIN OVER FOLIN-WU
2.3	1.9	.4	8.2	7.2	1.0
2.9	1.8	1.1	8.3	6.7	1.6
2.9	1.9	1 0	9.1	8.5	.6
3.0	2.5	.5	9 2	8.3	.9
3.1	2.1	1.0	9.2	8.3	.9
3.3	2.0	1.3	9 5	8.7	.8
3.6	1.9	1.7	9.5	8 9	.6
3.7	3 6	.1	9.5	6 7	2 8
4.0	3.3	.7	9.7	6 7	3.0
5.3	3.7	1.6	9.7	6 7	3.0
5.4	5.0	.4	10.0	9.1	.9
5.8	5.2	.6	10.2	8.8	1.4
6 1	4.2	1.9	10.7	8.3	2.4
6.1	4 8	1.3			

Folin[9] suggests preliminary precipitation of uric acid by means of silver lactate (his check method), in order to make sure that no significant portion of the blue color is due to substances other than uric acid. Table IV gives comparative results obtained by Folin's direct and check procedures. In all determinations results by the direct method exceed those by the check, though the differences are not so great as between the direct and Folin-Wu methods (Table II). It is evident that in our hands and in the bloods with which we have worked, values obtained by use of the new direct procedures of Benedict and of Folin are considerably higher than those obtained by the longer method of Folin and Wu. We cannot attempt explanation of

TABLE III

CONCENTRATION OF URIC ACID IN BLOOD BY THE METHODS OF FOLIN (1922)
AND BENEDICT (1922)

MG. PER 100 C.C. WHOLE BLOOD					
FOLIN (1922)	BENEDICT (1922)	EXCESS OF FOLIN OVER BENEDICT	FOLIN (1922)	BENEDICT (1922)	EXCESS OF FOLIN OVER BENEDICT
3.3	3.4	−.1	4.7	4.4	.3
3.5	3.5	0	4.7	4.6	.1
3.8	3.6	.2	4.8	4.5	.3
3.8	3.7	.1	5.0	4.6	.4
3.8	3.7	.1	5.7	4.7	1.0
3.8	3.6	.2	6.2	6.2	0
4.0	3.7	.3	6.3	6.8	−.5
4.0	3.8	.2	6.4	6.7	−.3
4.2	4.0	.2	6.8	7.0	−.2
4.4	4.1	.3	7.0	6.7	.3
4.5	4.0	.5	7.3	7.0	.3
4.5	4.2	.3	7.7	7.3	.4
4.5	4.6	−.1	8.2	7.0	1.2
4.6	4.0	.6	8.4	8.9	−.5
4.7	4.1	.6	10.4	9.4	1.0

TABLE IV

CONCENTRATION OF URIC ACID IN BLOOD BY DIRECT AND BY CHECK METHODS OF FOLIN (1922)

DIRECT	CHECK	INCREASE OF DIRECT OVER CHECK	DIRECT	CHECK	INCREASE OF DIRECT OVER CHECK
2.9	2.5	.4	6.2	5.2	1.0
3.0	2.8	.2	6.2	5.0	1.2
3.1	2.1	1.0	6.7	5.7	1.0
3.3	2.2	1.1	6.7	5.5	1.2
3.6	1.9	1.7	6.8	5.0	1.8
4.1	3.0	1.1	6.8	6.2	.6
4.3	3.6	.7	7.1	6.2	.9
4.3	4.1	.2	7.6	7.2	.4
4.5	4.2	.3	8.0	8.0	0
4.6	3.8	.8	8.5	7.3	1.2
4.9	3.6	1.3	8.7	7.6	1.1
5.0	4.3	.7	8.9	7.8	1.1
5.3	4.3	1.0	9.0	8.6	.4
5.5	4.3	1.2	9.2	6.5	2.7
6.1	5.2	.9	9.5	9.4	.1
6.1	4.7	1.4			

these differences, or present evidence as to which method, if any, gives correct measurement of the uric acid in blood. The use of various methods which yield discordant results adds greatly to the difficulties of clinical studies. It is to be hoped that conclusive evidence will be presented of the correctness of one or the other procedure for measuring uric acid in blood.

SUMMARY

It seems probable that the upper limit of normal for the new direct method of Folin may be 4.5 or 5.0 mg. per 100 c.c. of blood.

The concentration of uric acid in blood is considerably but inconstantly higher when measured by the direct methods of Benedict or of Folin (1922) than when measured by the method of Folin-Wu (1919) or by the check method of Folin.

REFERENCES

[1]Folin, O., and Denis, W.: A New Colorimetric Method for the Determination of Uric Acid in Blood, Jour. Biol. Chem., 1912-13, xiii, 469.

[2]Folin, O., and Wu, H.: A System of Blood Analysis, Jour. Biol. Chem., May 1, 1919, xxxviii, 81.

[3]Benedict, S. R.: The Determination of Uric Acid in Blood, Jour. Biol. Chem., March, 1922, li, 187.

[4]Folin, O.: A System of Blood Analysis, Revision of Method for Determining Uric Acid, Jour. Biol. Chem., October, 1922, liv, 153.

[5]Folin, O.: Nonprotein Nitrogen of the Blood, Physiol. Rev., July, 1922, ii, 460.

[6]Brown, H. and Raiziss, G. W.: The Estimation of Uric Acid in the Blood, Jour. Lab. and Clin. Med., November, 1922, viii, 129.

[7]Lennox, W. G., O'Connor, M. F. and Wright, L. H.: Studies of the Metabolism in Epilepsy. I. The Nonprotein Nitrogenous Constituents of the Blood, Arch. Neur. and Psych., January, 1924, xi, 54.

[8]Lennox, W. G.: Increase of Uric Acid in the Blood During Prolonged Starvation, Jour. Am. Med. Assn., February 23, 1924, lxxxii, 602.

[9]Folin, O.: Laboratory Manual of Biological Chemistry, New York, D. Appleton & Co., 1922.

ORGANIC, PROTEIN AND COLLOIDAL SILVER COMPOUNDS*

IV. Deterioration of Solutions on Keeping

By Torald Sollmann and J. D. Pilcher, Cleveland, Ohio

IT is currently believed that solutions of the organic silver compounds deteriorate rapidly on keeping, but we are not familiar with any quantitative data for this belief; and indeed it does not appear quite clear whether the assumed deterioration is in the direction of diminution or increase of the silver-ion concentration. The former would mean a decrease of antiseptic efficiency; while decomposition of colloidal into ionic silver would increase the irritation, but would also increase the antiseptic efficiency.

TABLE I

AGING OF SILVER SOLUTIONS ON THEIR ANTISEPTIC ACTIVITY

The figures represent the average inhibiting dose, i.e., the quantity of the compound, in milligrams per 10 c.c. of mixture, required to prevent gas formation by yeast under standard conditions.

AGE OF SOLUTIONS:	ONE DAY	ONE WEEK	ONE MONTH	TWO MONTHS	FOUR MONTHS	EIGHT MONTHS	ONE YEAR
In water							
Silver nitrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Protargol	2.2	2.6	2.7	3.1	3.1	3.5	5.0
Argyrol	66.	42.	21.	21.	17.	25.	30.
Silvol	93.	70.	61.	65.	60.	89.	—
In 0.9% sodium chloride							
Silver Nitrate	20	20.	20.	20.	20.	20.	20.
Protargol	63	80	48	68	>88	>140	>200
Argyrol	—	105	<49	29	48	37	
Silvol	140	>250	90	84	90	>145	

TABLE II

THE ANTISEPTIC EFFICIENCY OF SILVER SOLUTIONS, EXPRESSED AS PERCENTAGE OF THE
EFFICIENCY OF THE FRESH SOLUTIONS

AGE OF SOLUTIONS:	ONE DAY	ONE WEEK	ONE MONTH	TWO MONTHS	FOUR MONTHS	EIGHT MONTHS	ONE YEAR
In water							
Silver Nitrate	100	100	100	100	100	100	100
Protargol	100	85	83	71	71	63	44
Argyrol	100	157	314	314	390	264	220
Silvol	100	133	153	143	155	104	—
In 0.9% Sodium chloride							
Protargol	100	79	131	92	<71	<45	<31
Argyrol*		100	>214	362	218	283	—
Silvol	100	<56	155	166	155	<96	

*Expressed in percentage of solutions a week old.

With the hope of supplying direct and quantitative answers to these
questions, we have applied the yeast method, to test the antiseptic efficiency,
and therefore the silver-ion concentration, of solutions of representative silver
compounds, kept for various periods of time.

The following solutions were used:

Silver nitrate	10%
Protargol,	10%, two specimens.
Argyrol,	10%, two specimens.
Silvol,	20%, three specimens.

The solutions were kept in ordinary bottles, cork stoppered, in diffuse
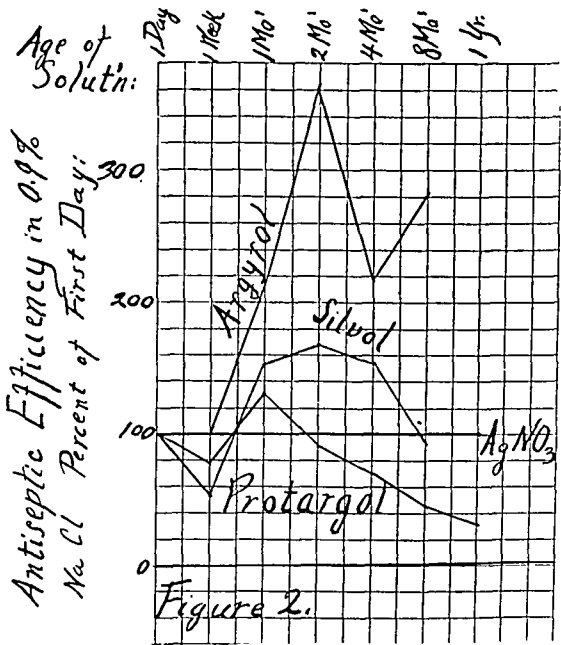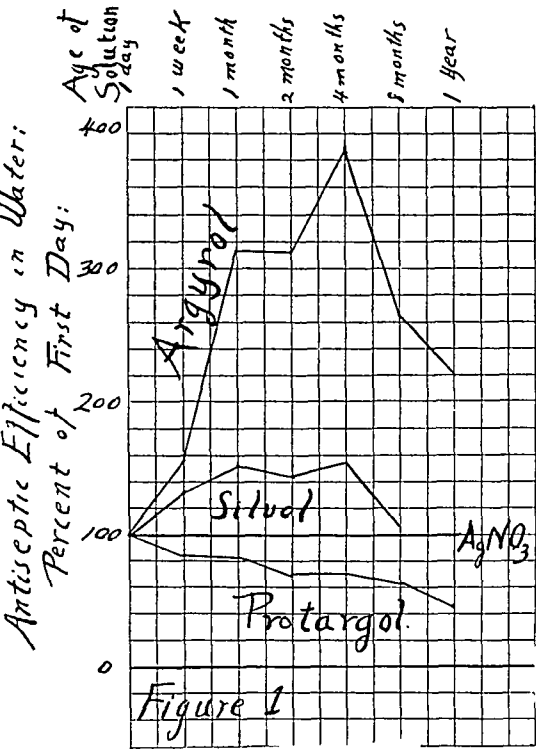daylight, without special precautions as to asepsis. The concentration re-

Fig. 1.

Fig. 2.

quircd to inhibit the growth of yeast was determined in water, and also in normal saline, by the method described in our previous papers, on the day when the solution was made, and again at the end of a week, and of 1, 2, 4, and 8 and 12 months.

Table I shows the averages of the dosage required to inhibit the growth of yeast.

The changes will be more easily appreciated if they are expressed in terms of the original antiseptic efficiency, as in Table II and Figs. 1 and 2.

SUMMARY

The graphs show at a glance that the organic compounds undergo progressive changes, which are quite marked within a week. They are fairly parallel for watery and saline media; but their direction is absolutely the reverse for protargol on the one hand, and for argyrol and silvol on the other hand:

1. Protargol (which may be taken as representative of the *Protargentum Fortius* type) *loses* progressively in antiseptic activity. Evidently the ionic silver (which constitutes about $\%_0$ of its total silver content) is gradually reduced to insoluble or otherwise inactive silver. The *activity is decreased,* in a month, to about $\frac{4}{5}$ of the original; in 8 months, to about $\frac{2}{3}$ of the original; in a year, to about $\frac{1}{2}$ of the original.

The loss, although considerable from the standpoint of percentage, would probably not have much clinical importance.

Argyrol and Silvol (representing the type of *Protargentum Mite*) *increase* in antiseptic efficiency, and therefore in irritation, with age. This is probably due to the progressive ionization of a part of their colloidal silver, perhaps through decomposition of their protective protein. The *activity and irritation are increased:* In a week, by about $\frac{1}{2}$ for argyrol, $\frac{1}{3}$ for silvol; in a month and onward, to about 3 times for argyrol, by about $\frac{1}{2}$ for silvol.

The changes within a week might cause sufficient irritation to modify the clinical response.

CONCLUSIONS

Solutions of the colloidal silver compounds show progressive changes of antiseptic efficiency on keeping.

Protargol solutions (and presumably others of the same type) become poorer in ionic silver, and therefore less efficient; but even in a year the change would not be of much clinical importance.

Argyrol and Silvol (and presumably others of the type), become richer in silver ions, and therefore more antiseptic, but also more irritant. The changes start rapidly, so that a week might suffice to modify the clinical response.

STUDIES IN BLOOD CELL MORPHOLOGY AND FUNCTION*

II. Morphologic Changes of the Blood in Myelogenous Leucemia under Radium Treatment

By Max Maurice Strumia, M.D., Philadelphia, Pa.

SINCE the work of Renon[1] and his coworkers in France, and Schueller[2] in Germany, the study and application of radium in the treatment of leucemia, especially of the myelogenous type, has been extensive; and, as is most widely admitted today, it constitutes the best known therapeutic agent against this disease.

The changes occurring in the circulating blood during radium treatment are of utmost importance, because they reveal, to a certain extent, the modifications which are going on in the foci of the leucemic tissue. The results and conclusions of some of these studies will be considered later. The purpose of this work is to present an accurate study of the changes occurring in the circulating blood of a patient of myelogenous leucemia during the radium treatment, together with some new observations on the relative behavior to radium of the various blood cells, in order that data may be gained to corroborate with biologic proofs the relationships of some of the blood cells.

Studies of such a nature have been rather numerous, yet the lack of detailed differential counts in some of them and irregular and perhaps inaccurate blood counts in others have vitiated the results of these interesting studies. Especially faulty has been the frequent massing of cells of entirely different origin, as primordial cells, myeloblasts, large lymphoblasts, etc., under the common name of mononuclear cells.

From an extensive review of the literature on the subject, it seems that roentgen rays and radium are fundamentally alike in their action on the leucemia, provided that the same quality of radiation is used. Radium is perhaps more constant and quicker in its effect (Gulland,[3] Ordway,[4] etc.).

Falta and his coworkers,[5] confirmed by Kauffman,[6] found that thorium and mesothorium are similar to radium in their effect on leucemia.

Various findings of investigators using roentgen rays will be promiscuously referred to, as well as findings of those using radium in any form or method.

CASE.—The patient, J. A., age forty-eight, white, male, has no points of interest in the family history, and in his remote anamnesis. About one year and a half prior to the time when first seen, the patient noticed a lump just below the margin of the ribs on the left side, which gradually increased in size. The patient did not feel any other

serious symptoms referable to the disease, except a vague sense of weakness, pallor, and loss of appetite.

The first physician from whom the patient sought advice administered a few intravenous injections of an arsenical preparation. The patient first came to our notice in the beginning of October, 1922, and at that time showed a rather high degree of anemia; the heart was slightly enlarged, and the first sound was accompanied by a very light, soft, blowing murmur. The blood-Wassermann was negative. The spleen reached five inches below the costal margin, and the liver was also palpable two and one-half inches below the costal margin. The blood count was: White cells—133,000; R. B. C. 4,450,000, Hb. 75. The differential count is given in Table III. This count is the average of three counts taken on three consecutive days, the last being taken October 10, 1922. The following day, radium treatment was started at the Radiological Department of Philadelphia General Hospital under the direction of Dr. R. Bradley, to whom I am greatly indebted for his valuable cooperation and for the account given as to the method of the radium applications.

In doing the blood examinations, the following precautions were used: the samples for blood counts were taken with the same pipettes, certified, and the counts were done with the same counting chamber, also certified, using the same technic, the same person doing all the counts.

The Hb. estimations were done with the colorimeter, using as a standard (100 per cent) a solution of dog's blood in N/10 HCl., containing hemoglobin in amount of 13.8 per cent. The preparation of the standard was done by determining the oxygen capacity of the dog's blood.

The slides were stained with May-Giemsa stains, using Pappenheim's technic,[7] and with the oxidase stain, using Goodpasture's[8a] method. The methyl-green-pyronin method was also employed in the differentiation of lymphatic cells. The differential counts were made on two separate slides, at different times, to avoid bias and self-suggestion, not less than 500 cells being enumerated. As a matter of fact, in 33 differential counts made, 24,846 cells were counted, averaging 753 cells at each enumeration. Enough emphasis cannot be laid upon the necessity of faultless technic in blood sampling, in order that the results be strictly comparable. The necessity of counting a very large number of cells is just as important, so as to obtain a true figure, especially when the variety of cells is large.

In recording differential counts, Ferrata's[8] classification, somewhat modified, was used. The blood counts were taken for the first few months possibly every other day, always in the morning before the radium application. After the first two months, at variable periods of time as indicated in Table I and III.

The coagulation time was taken approximately every month. The radium was applied in form of emanations, using the method of pack, the radiating tubes being at a distance of six inches from the skin and filtered so as to leave free course to the penetrant gamma rays. Table IV gives details of the radium applications.

We will now analyze the blood picture following radium application.

White Cell Count, Red Cell Count and Hemoglobin.—It will be seen from Table I, that from the first application or two, both leucocytes and erythrocytes greatly increased in number; the white cells from 133,000 to 202,700 and the red cells from 4,450,000 to a maximum of 7,040,000. This considerable increase is without doubt due to the stimulation or irritation of the first doses of radium, as was confirmed by changes in the differential count and by the very peculiar increase of the nucleated red cells, especially of the megaloblastic type, as will be discussed later.

The white cells, *after the first momentary rise*, steadily decreased under the effect of radium; then they oscillated for about a month between 17,000 and 40,000 and finally went down to normal. The downward course of the white cells continued also after the radium applications were discontinued, reaching a minimum of 5,000 about three months after the last dose of radium. This indicates the advisability of stopping the radium application as soon as the white cells are near normal, between 10,000 and 15,000. Gulland[3] also found that the effect of radiation goes on for a while after the termination of the treatment.

The last applications of radium were given on December 20, 1922, the white cells numbering about 6,000. They started to increase again in June, 1923, six months later, and then they rapidly increased, reaching 36,000 at the end of July, 1923, and 75,000 on August 29, 1923.

The red cells after the temporary increase were no longer affected, so far as the count is concerned.
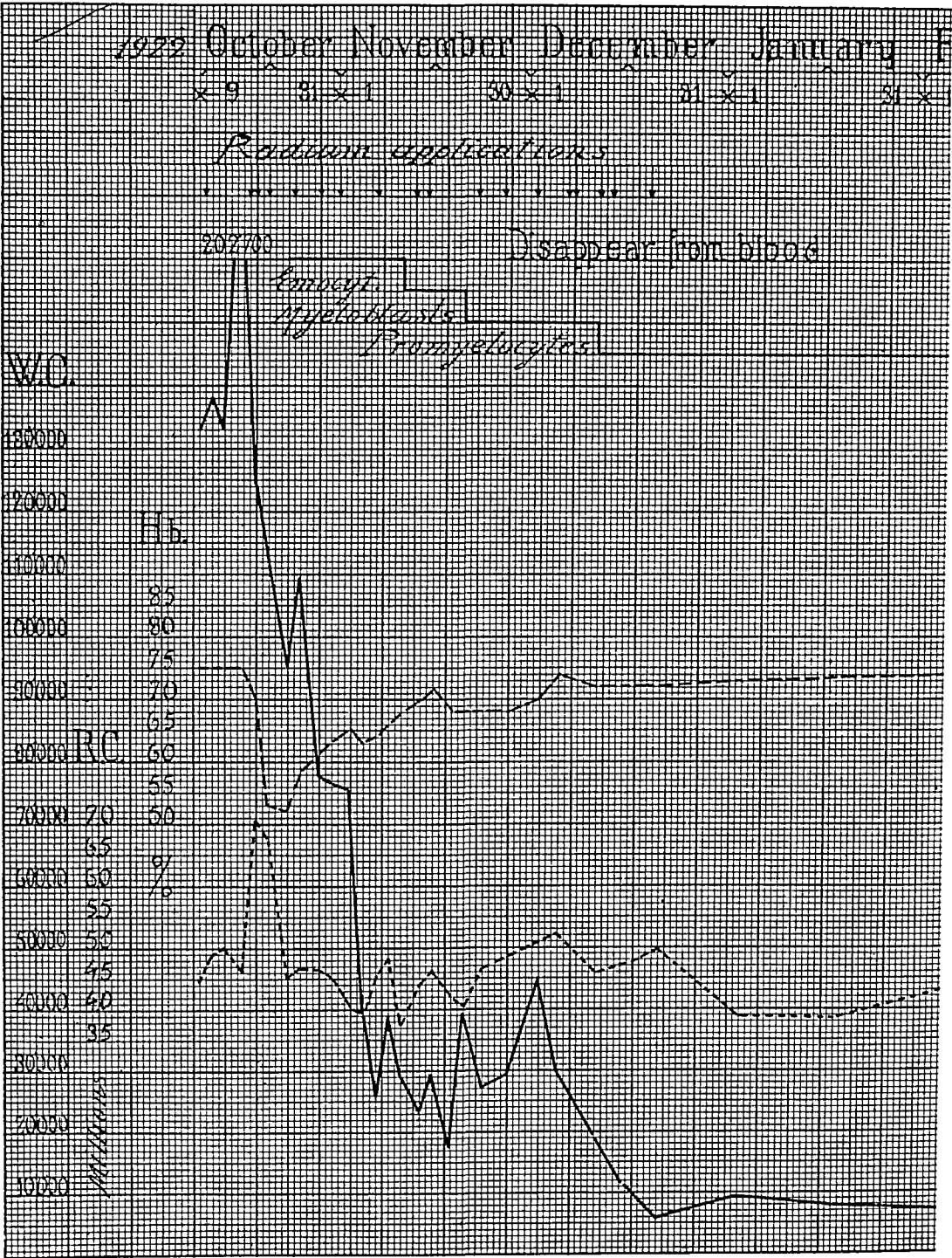
Table I.—Variations of the blood counts and the hemoglobin percentage; this diagram also shows order in which immature cells disappear from the blood. The radium applications are recorded in the upper portion.

The hemoglobin, during the period of rapid increase of red cells, decreased from 75 per cent to 52 per cent in seven days, which testifies to the irregular production of red cells under the stimulus of radium. After this drop, the hemoglobin slowly rose, reaching a percentage of 85, as compared to 75 per cent previous to treatment.

During the last month of observations, August, 1923, the hemoglobin decreased to 70 per cent again, while the leucocytes rose from 26,000 to 75,000 in twenty-nine days.

Table I—Cont'd

It is therefore obvious that the curves of the white cell counts and the percentage of hemoglobin run an exactly contrary course.
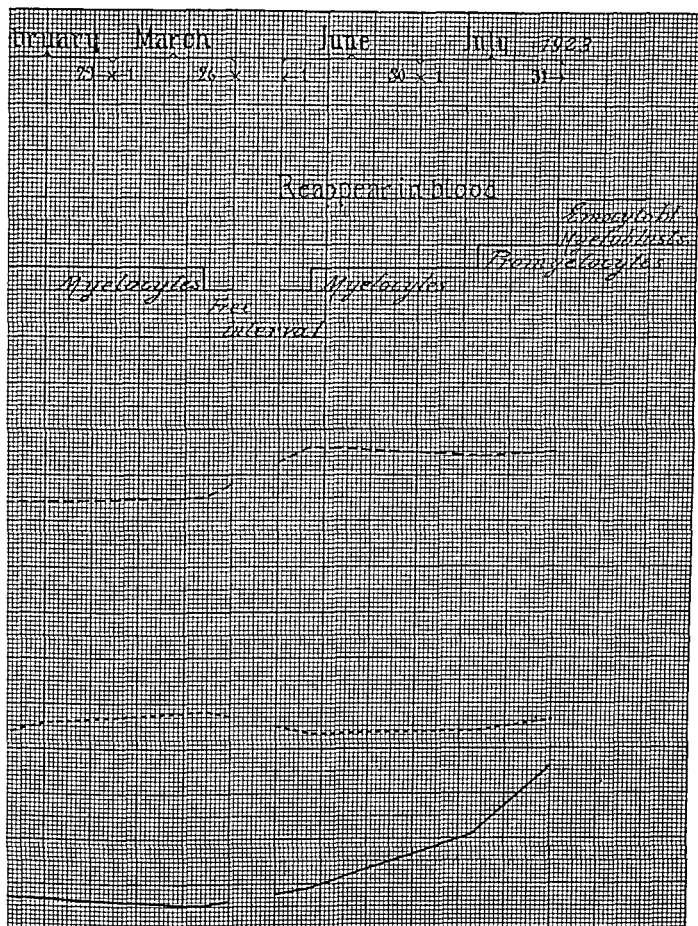
Primordial Cells.—The primordial cells, or hemocytoblasts (Ferrata[8]), which should be identified with Pappenheim's lymphoidocytes, after a short period of stimulation in which they were slightly increased, rapidly and steadily decreased until they totally disappeared a month after the beginning of the treatment, or after ten radiations had been given, totalling 46,400 millicurie hours.

The order in which the immature cells disappeared from the blood remarkably bears out their genealogic relationship; in fact as it is shown in the upper part of Table I, the first cells to disappear were the primordial cells, which in all probability are the undifferentiated mother cells of all the cellular elements of the blood; then the myeloblasts; the promyelocytes and finally the myelocytes, all belonging to the granulocytic series.
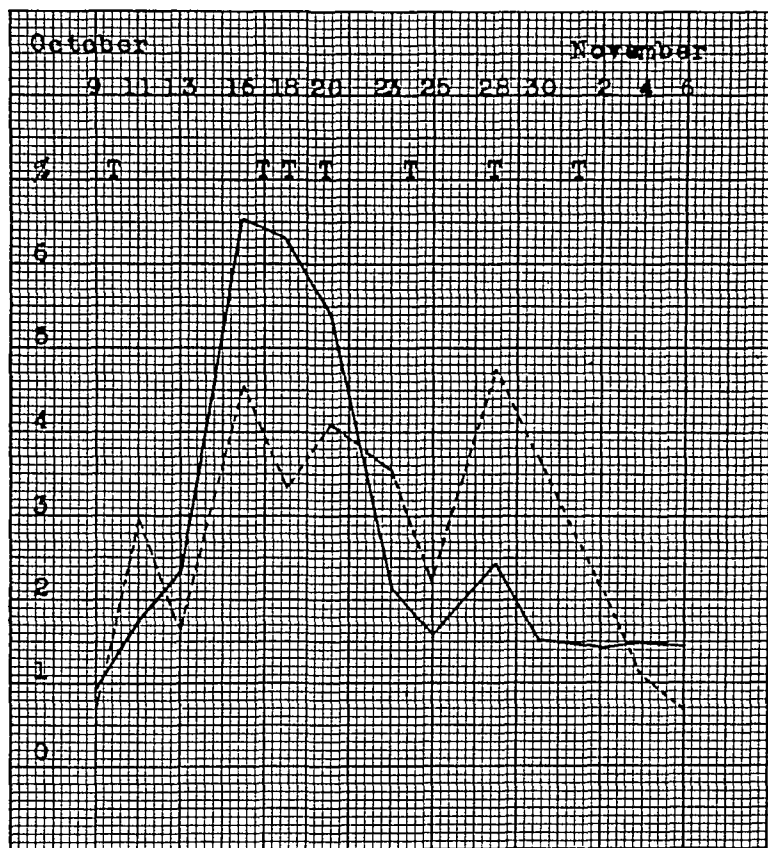

Table II.—The stimulating effect of the first radium applications on the immature and pathologic cells of the hemoglobinic series.

————: Pathologic cells of the hemoglobinic series.
......: Immature cells of the hemoglobinic series.
    T: Radium treatment.

In contrast, the primordial cells were the last cells to reappear when the white cells increased again, six months after the last radium application.

Immature Cells of the Leucocytic Series.—Myeloblasts, promyelocytes and myelocytes disappeared in the order mentioned. The metamyelocytes were the only cells of the series present, in small number, after the radium treatment, which testified that the pathologic activity of the leucemic centers was only greatly attenuated, not entirely destroyed by the radium. The period of time in which immature cells of the leucocytic series were almost absent from the blood and in which red cells and hemoglobin were normal is indicated in Table I, as "free interval." This lasted about three months, starting about seven weeks after the last radium treatment was given. During this interval the size of the spleen was within normal limits and the patient's general condition good.

Several blood counts and differentials were conducted during this period, but the variations were of no importance and are not reported in Table I. During the period of treatment with radium, the relative number of mast cells was apparently increased, (Whitcher[9]).

The considerably longer time required by the myelocytes to disappear from the blood as compared with the other immature cells of the granulocytic series (myeloblasts, promyelocytes), is due to the fact that the myelocyte is an adult form which in order to become a normal polymorphonuclear does not require anything but an ontogenetic ripening; (Ferrata[8]). In other words, as to the effect produced by radium, the granulocytic cells are sharply divided in two classes; cells having totally (myeloblasts) or partially (promyelocytes) basophilic protoplasm, which are readily affected by radium, and cells with neutrophilic protoplasm (myelocytes, metamyelocytes, polymorphonuclears) which are less easily affected by the radium.

Myeloblasts, and promyelocytes are, as a matter of fact, the cells whose presence in the circulating blood, together with other symptoms, has an almost pathognomonic value for the diagnosis of myeloid leucemia, because these cells are very seldom found in other conditions, while small numbers of myelocytes are found in many other diseases, when the hemopoietic organs are more or less involved.

The effect upon the immature cells of the lymphocytic series will be considered later.

Immature Cells of the Hemoglobinic Series.—During the days following the first two radiations, the number of the nucleated red cells increased considerably, together with the number of the pathologic red cells as shown by Table II. This increase, evidently due to a stimulation from the radium, is even more interesting considering that it acted to a great extent upon the megaloblastic elements which were considerably increased after the first application. In the blood smears taken these days, the number of megaloblasts and normoblasts in mitosis was surprising, a sign of great activity in the erythropoietic centers.

Following this rise, the number of the nucleated red cells rapidly decreased under the effect of large doses of radium, the megaloblasts being the first to disappear, adding another point to the theory that the less mature the cells, the more quickly they are affected by radiation. Nucleated red cells of all kinds and pathologic red cells entirely disappeared during the so-called "*free interval*," to reappear with the renewed activity of the hemopoietic tissue, when the white cells increased again in number. This similar behavior of the red and white cells bears out the relationship between affections of the erythropoietic and of the leucopoietic system. Perhaps an illustration of this is found in the disease, or better syndrome, known under the name of Von Jaksch's,[10] or pseudoleucemic anemia of childhood.

Pathologic Cells of Leucocytic Series.—These were also increased slightly, absolutely as well as relatively, after the first few doses of radium, especially the Türk and Rieder cells, (degenerative forms) but soon under the effect of subsequent doses of radium, they entirely disappeared from the circulation. (See Table III.)

Platelets and Coagulation Time.—In another case of leucemia being treated with radiation, we noted the increasing facility with which the blood would flow from the finger tip as the treatment was being given. In this case, this phenomenon was also noted. The coagulation time, which was taken every month or so, before treatment was about two minutes. During the treatment the coagulation time steadily increased and was six minutes during the "*free interval*"; then during the last two months of observation, while the white cells were going up again, the coagulation time was reduced to three and one-half minutes. The platelets and their progenitors, the megakaryocytes, followed exactly the same course: the megakaryocytes disappeared from the peripheral blood after eight applications of radium in a period of twenty days, and the platelets, which were very numerous in the first blood films, diminished considerably until they were reduced to normal number in about two months after treatment was begun. Giffin[11] has also reported diminution of the number of platelets following protracted radiation treatment.

Mature Leucocytes.—The absolute count of the polymorphonuclear neutrophiles went steadily down from 73,788 per cmm. before the treatment, to a minimum of 2,761 per cmm. during the *"free interval,"* to climb again during the last two months. The relative count, however, increased during the treatment from 55.48 per cent to a maximum of 86.04 per cent, which was reached when 19 treatments of radium out of the total of 20 had been given.

The relative count of the polymorphonuclears stood about 80 per cent during the last period of the treatment, while the absolute count of the mature lymphocytes was around 800 per cmm. and their relative count was about 10 per cent.

A few days after the treatment of radium was stopped, the relative count of the

		DATE	10/9	10/11	10/13	10/16	10/18	10/20	10/23	10/25	10/30
PRIMORDIAL CELLS		Hemocytoblasts	.69	.12	.28	.37	.82	.22	.32	.31	.34
IMMATURE CELLS OF THE LEUCOCYTIC SERIES	Myeloblasts	With small azurophile granules	1.95	.47	.47	.93	.32	.11	.32	.31	.81
		With large azurophile granules	.20	.18	.20	.37	.11	.11	.42	.13	.17
	Promyelocytes.f...	With small azurophile granules	2.40	8.75	4.31	2.15	2.73	1.95	4.60	1.23	2.06	2.80
		With large azurophile granules	.57	.74	.20	2.34	1.43	.72	.30	.93	.51	.20
	Myelocytes	Neutrophiles	13.65	16.53	15.72	17.17	14.66	14.38	11.60	24.09	23.63	51.20
		Eosinophiles	.13	.40	.28	.37	.16	.11	.20	.05		.20
		Mast	.60	.47	.10			2.48	.70	.62	2.06	.25
	Metamyelocytes	Neutrophiles	13.67	6.47	9.10	7.60	20.21	7.44	4.73	9.20	5.00	5.70
		Eosinophiles						.16	.11			
		Mast				.20	.19	.32			
MATURE LEUCOCYTES	Monoblasts			.22	.27	.08					
	Lymphoblasts		1.72	1.58	2.46	2.06	2.41	1.55	.82	1.49	1.21	1.30
	Prolymphocytes		1.60	.17	.10	.74	.54	.41	.30			.16
	Granulocytes	Neutrophiles	54.13	60.40	59.96	58.72	45.00	61.66	64.33	51.66	51.21	59.33
		Eosinophiles	.47	.12		.20	.16		.40		.27	.20
		Mastleucocytes	.23	.60	.66	.23	.25	.72	1.20	.93	.98	1.10
	Monocytes		.48	.36	.77	.36	.41	.21	.32	.32	.85	1.10
	Lymphocytes		4.70	3.10	2.46	3.00	5.64	2.74	2.66	1.87	3.50	3.54
	Leucocytoid lymphocytes		5.34	2.76	3.20	6.53	4.18	1.10	6.60	9.00	3.00
IMMATURE CELLS OF THE HEMOGLOBINIC SERIES	Megaloblastic	Promegaloblasts		.09				.09		.15	.08
		Megaloblasts,basophilic	.43	.09	.17	1.07	.37	.56	.44	1.10	.62
		Megaloblasts,polychromatophilic	.05	.43	.46	.54	.44	.47	.47	.66	1.12	.62
		Megaloblasts,orthochromatic	.33	.13	1.22	.37	.27	.44	.31	.31	.31
	Normoblastic	Proerythroblasts							.14	.19	.50
		Erythroblasts, basophilic	.43		.37	.47	.47	.14	.47	.1?
		Erythroblasts, polychromatophilic	.66	.12	.46	1.03	.44	.91	1.22	.90	.94	.70
		Erythroblasts, orthochromatic	.21	1.25	.37	1.90	.62	1.47	.74	.42	.15
PATHOLOGICAL CELLS OF THE LEUCOCYTIC SERIES	Rieder type		.21			.16	.22	.34
	Turk type		.10		.20	.33	.62	.20	.10
	Plasmazellen		.05		.10				
	Megacaryocytes		.02	.09	.16	.56	.47	.62	.16	.17
PATHOLOGICAL CELLS OF THE HEMOGLOBINIC SERIES	Polychromatophilic normocytes		.71	.95	1.74	6.18	3.13	1.60	1.42	.73	1.41	.90
	Normocytes with basophilic degeneration		.24	.54	.37	.09	.09
	Normocytes with Jolly bodies				.37	.19	.09	.14	.12	.17
	Normocytes with Cabot rings		.12		.09
	Megalocytes		.23	.80	.64	.80	2.30	3.37	.66	.73	1.88	.80
TOTAL CELLS COUNTED			2604	838	1044	1066	1225	967	1000	643	802	1170

Table III.—Differential counts taken through the period of observation.

polymorphonuclears started to diminish, reaching a minimum of 55.22 per cent, with an absolute number of 2,761 on March 20, 1923, during the *"free interval."*

The lymphocyte count, both absolute and relative, steadily climbed after the treatment was stopped, and on March 20, their relative count was at the maximum of 37.23 per cent, while the absolute number was 1861 cells per cmm., that is a little over the normal.

Summarizing: during the treatment the polymorphonuclears diminished in the absolute number, but their relative number went up; lymphocytes diminished in the absolute and relative number. Immediately after treatment, the number of polymorphonuclears, both relative and absolute, went down; while the lymphocyte number, both absolute and relative, went up. This tends to show that apparently lymphocytes pick up very easily as soon as the radium treatment is discontinued, while the radium action on the mature granulocytes goes on for a longer period. This point will be further discussed later.

total number of cells and even in the disappearance of certain groups of cells. If we assume that the leucemic process is uniformly diffused in the body or at least in most of the hematopoietic centers, that is, in the bone marrow, then by radiating only a portion of the leucemic tissue, a localized and not generalized reaction should be obtained. Or, stated in another way, a quantitative and perhaps qualitative variation might be expected, but not the disappearance of all the cells of a certain type, and this was exactly the case, because long before all the bones of the lower extremities were entirely radiated, leaving untouched all the rest of the skeleton, the primordial cells disappeared entirely from the circulating blood. Therefore it is a reasonable assumption that radium acts upon the leucemic tissues in a generalized manner. Russ[25] found that doses of radium applied locally in malignant cases had a general effect on blood.

It is apparently of little significance where the radium is applied, because the entire body shares a large amount of penetrant rays; of most importance is the quality of the radiations.

The manner in which the generalized action by radium is produced, does not lend itself to a definite conclusion. We offer as a possible explanation a production of leucotoxic material by the disintegration of the cells directly affected by the radiations, in the circulating blood, or in the leucemic foci of the region radiated. This material, circulating in the blood stream, may be the cause for such a generalized alteration of hematopoietic centers. It may be possible that the blood cells and blood plasma, perhaps, carry the radium emanations from the point of application to all the leucemic centers, thus bringing about the destructive changes. We offer these explanations merely as possibilities.

That limited doses of radium produce a stimulating effect on all hemopoietic centers is borne out by the increase in the number of leucocytes and erythrocytes; and by the increase in the number of immature (nucleated) red cells, especially of the megaloblastic type, observed after the first few radium applications. The same results were observed in two other cases of myelogenous leucemia, treated with radium. Such stimulating action is of the greatest importance, because it might be made the base of the treatment of primary anemia by the use of small doses of radium.

Gulland,[3] in England, did not derive satisfactory results from radium applications in pernicious anemia, but admitted the possibility of utilizing the stimulating effect of radiations with the exclusion of the destructive. Otto Neu[17] favorably treated pernicious anemia with x-rays. Studying the table accompanying the report of the case of leucemia treated with radium by Whitcher,[9] it appears that following the first radium application, the white cells that were 164,800 before treatment, after a momentary drop to 135,000, increased to 312,000. Lazarus Barlow,[18] experimenting on rats, cats, rabbits, and frogs, found an increase of polymorphonuclears and erythrocytes, as a result of what he calls shock radiation. Barlow in animals[18] and Otto Neu[17] also found an increase of red cells in pernicious anemia patients under the effect of radiations. An increase of hemoglobin and erythrocytes

of leucemic patients submitted to protracted radium treatment has been observed by most of the authors who studied the subject. Such an increase is always parallel to a decrease in the number of the white cells.

Whether this increase is due to a stimulation of the erythropoietic tissue, or simply to a betterment of the conditions of lodging of the erythropoietic nests in the bone marrow, due to the reduction of the granulocytic elements, or to both, as we believe, is not easily understood. Gulland[3] puts the same question but is unable to answer it. Increase in the number of nucleated red cells following radiation is reported by Peabody[12] and a study of the tables of Schisler[13] shows that the same thing happened in his case. The stimulation of erythropoietic tissue by radium is especially marked in the megaloblastic or embryonic centers. Otto Neu,[17] treating cases of pernicious anemia, found megaloblasts in the blood which had not been present before the treatment. Price-Jones,[19] studying the effect produced by radiations in the bone marrow by exposing rabbits to five grams of radium bromide, found a definite increase in the percentage of the megaloblasts, and metrocytes. The author regards the result as due to a combination of white cell destruction, and diminished formation of granular leucocytes, with an exaggerated production of red cells. Furthermore anemia produced by radiation is often of the primary or pernicious type with megaloblasts (Gulland,[3] Giffin[11]). Cases of fatal anemia from radium are reported by Mottram,[20] Bordier[30] and others. All cases were of the aplastic type, accompanied by considerable leucopenia.

A much argued point is whether the lymphocytes of the circulating blood are more affected than the mature granulocytic cells by radium. Heinecke,[21] as the result of experimental studies, was the first to report an eclectic action of radiation upon the lymphoid tissue of the spleen, lymph nodes and bone marrow. Murphy and Ellis[22] found that by carefully regulated doses of x-ray repeated at intervals, gradual atrophy of the lymphoid tissue may be accomplished, without any appreciable effect on the other tissues.

Mottram[23] concludes from his experiments, on rats, with gamma radiations that bone marrow is less affected than lymphoid tissue. This was found true also for soft x-rays and beta rays of radium by Mottram and Russ.[24] Russ,[25] experimenting on patients suffering from malignant diseases where the body was sharing to a large extent the gamma rays, observed reduction in the number of circulating lymphocytes in twenty out of thirty-one cases studied, increase in five and no changes in six. Anything less than an 8 per cent variation was classified as no change. The figures for the polymorphonuclears in the same thirty-one cases, showed that an increase, a decrease, and no change in their number, occurred in almost the same amount. In another study, Russ and his coworkers[26] came to the almost paradoxical conclusion that the lymphocytes in the circulating blood of the rat were more sensitive to x-rays than a photographic plate. Mottram[27] found a drop in lymphocytes and polymorphonuclears in x-ray workers. The number of lymphocytes and polymorphonuclears is low in radium workers also. The

diminution in the polymorphonuclears is more marked than in x-ray workers.

Lazarus-Barlow,[18] in his experiments with rats, using five grams of radium, found that the destruction of lymphocytes is the most characteristic effect. Warthin,[28] in an experimental study on the effect of x-ray upon blood-forming organs, found a marked effect upon the lymphoid tissue of the spleen and lymphoid glands, the small lymphocytes being the first to be affected. In contrast with the finding of Russ, Mottram and the others on the subject, great reduction of lymphocytes in rats after a short exposure to radiations is not the experience and conclusion of Leitch.[20] The point was made that it is not the specific action of the x-ray, but rather the effect of the fright suffered by the nervous rat on the exposure to the noisy x-ray room. The author also failed to find any reductions of lymphocytes in the blood of five patients who were undergoing x-ray treatment.

Ordway[4] reports relative lymphocytosis after treatment; in one case reported the number of polymorphonuclears increased from 60 per cent to 78 per cent, and then diminished to 50 per cent. The absolute number fell from 299,475 to 2,917. The relative number of lymphocytes rose from 2.3 per cent to 45.7 per cent, and the absolute number of lymphocytes fell from 11,750 to 2,650. Peabody[12] found after treatment a larger proportion of mature polymorphonuclears. In one of the cases reported by Renon, Degrais and Tournemelle,[31] it is interesting to see that, notwithstanding the great reduction of white cells, the total number of lymphocytes per cubic millimeter was still 941 proportionately higher than the number of polymorphonuclears, 1,705 per cubic millimeter. Schisler[13] found a continuous relative increase of polymorphonuclears under x-rays and benzol, and a decrease in the absolute number of small lymphocytes, but no effect upon the relative count.

Whitcher,[9] in reporting a case of leucemia treated with radium which ended fatally, states that in the final period the polymorphonuclears had fallen to 4 per cent, while the lymphocytes had risen to 58 per cent. The blood picture resembled that of lymphatic leucemia. In one of the cases reported by Oppenheimer,[14] treated with x-rays, death occurred with a transition of the myelogenous leucemia into an acute lymphemia.

In our case the reduction of the absolute number of the lymphocytes was proportionately much smaller than the reduction of the polymorphonuclears, the latter being reduced to approximately one-twenty-fourth of the original number, the former only to one-sixth. Giffin[11] found that radium caused an increase in the percentage of small lymphocytes, after the reduction of the total leucocyte count; but a reduction of the absolute number to approximately one-tenth of the original number of cells.

The leucocytoid lymphocytes, which we found to be markedly increased after radium applications, may be considered in the light of our findings as partially degenerated lymphocytes. They are probably similar to those found by Warthin[28] in the peripancreatic and mesenteric lymph nodes of mice submitted to x-rays and in the spleen and lymph nodes of a patient of leucemia treated with x-rays.

A marked difference is referable to the immature cells of each series; the immature cells of the granulocytic series (myeloblasts, promyelocytes, myelocytes and metamyelocytes) are more readily and decidedly affected than the immature cells of the lymphocytic series (prolymphocytes and lymphoblasts). The lymphoblasts and prolymphocytes therefore act more like mature cells and are considerably more resistant to the radium action. This probably explains the less effective results obtained in chronic lymphatic leucemia when treated with radiations.

In a case of chronic lymphatic leucemia submitted to few radium applications, the number of small lymphocytes, lymphoblasts, and prolymphocytes was proportionally diminished in the same way. This result apparently contrasts a little with the supposition that lymphoblasts and prolymphocytes are to the lymphocytes what promyelocytes and myelocytes are to the polymorphonuclears (Ferrata[3]). In other words, genealogically speaking, as far as the radium effect is indicative, a mature lymphocyte is nearer to the prolymphocyte and lymphoblast than a polymorphonuclear is to the myelocyte, promyelocyte and myeloblast. Summing up, the circulating lymphocyte of patients of leucemia under radiation treatment has been found by most authors to increase in percentage and to diminish in the absolute number.

In our case, the latter show a considerable and constant decrease, but stated in terms of percentage an increase occurred in the first part of the treatment and was followed by a decrease which continued even after the radium was discontinued. The experiences of Heinecke, Murphy and Ellis, Russ, Mottram, etc., on rats, reported before, have met with general approval, the only dissension being on the part of Leitch. At any rate, before any far-reaching conclusions are drawn from such reported sensibility of the circulating lymphocytes of rats to radiation, as to the mechanism of lymphocytes in the protection against diseases (Murphy and Ellis,[22] Murphy and Morton[32]), the problem should be further studied in the human being.

The order of disappearance of abnormal cells from the blood stream bears a direct relation to their immaturity or, expressed in other words, the more embryonic the cell, the easier it is affected by radium. This has been the experience of most experimenters in similar studies. We consider this phenomenon evidence which supports the conception of the histogenesis of the granulocytes from the undifferentiated or primordial cells; and of each granulocyte springing from the cell immediately above it in the granulocytic series. Further evidence of this is offered by the recurrence of the immature cells in the circulating blood (when radiation has been discontinued) in inverse manner to their disappearance. This behavior opposes the assertion of Gulland[5] that radiations have much less effect on tissues of embryonic or undifferentiated type, than on those more fully developed. Ordway[4] found the immature forms to be more affected by radium than the mature ones; in Peabody's[12] experience, under radium treatment, myelocytes and immature forms became less prominent. All this goes to corroborate the view that the more immature the cell is, the less resistant it will be to the destructive effect of radium.

It is imperative, as emphasized by Peabody and others, that the patient under radium treatment receive constant supervision. Periodic white cell count should be made, not only during radiation, but particularly after radiation, because the fluctuation in the number of leucocytes is usually parallel to the variation of the general conditions of the patient, and sometimes precedes them.

The results here presented were confirmed in every point by the study of another case of chronic myelogenous leucemia, L. R. J., an adult negro, treated with radium in exactly the same manner. In this case, which was more advanced than the other just reported, the white cell count was 390,000 on February 3, 1922. The radium treatment was started on February 4, 1922, with the same technic indicated in the other case. The first series of treatment, 14 applications with a total of 76,516 Mc. hours, ended on March 12, 1922; on March 14, the white cell count was 42,000. On March 24, the white cell count was 10,600, this further drop in the number of leucocytes confirming the view that the action of the radium goes on for a while after its applications have been discontinued. The white cell count stood near normal for about a month and then slowly rose and was 30,400 on June 11. A new series of radium applications was started on June 16, this time numbering eleven, with a total of 45,616 Mc. hours, the last application being given on September 5, 1922. This new series of applications again brought the number of white cells near normal and they were 11,700 on November 20, 1922. These results were obtained from the record cards of the patient. In the beginning of February, 1923, we started a series of more detailed blood counts on the patient. On March 14, the white cell count was 73,100; on the seventeenth a radium application of 7,416 Mc. hours was given, and on the following day the number of white cells was 116,210. In the blood films taken on that day the nucleated red blood cells were very numerous, especially those of the megaloblastic type. This confirms that the action of the first dose or doses of radium have a decidedly stimulating action upon the blood-forming centers. The treatment was then continued, but the effect of this new series of treatment on the leucocytes, although showing fundamentally the same tendencies, was considerably less marked, probably due to a certain degree of resistance gained by the leucopoietic tissue against the radium.*

The reduction of myelocytic cells in the circulating blood, both relatively and absolutely, is perhaps the most constant and important effect of the radium treatment, because it runs parallel to the reduction of the total white cell count, the gradual reduction of the spleen and to the betterment of the general condition of the patient.

The early experimenters, as well as many others, report this striking reduction of the myelocytes after radium treatment (Renon, and Degrais, Giffin, Ordway, Peabody, Schisler). Whitcher also reports a very large diminution of the myelocytes, and besides in studying his tables, one notes a "free interval" similar to that studied here. The last treatment of the third series of radium applications was given to his patient September 9, 1920.

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*Follow up note: The patient died September, 1924, the autopsy amply confirming the diagnosis.

Myelocytes were then present. They disappeared at a later date, without new radium applications, and they were not present October 7, but they reappeared on October 28, 1920. The same thing was repeated during other periods of the treatment.

CONCLUSIONS

1. The effect on the morphology of the blood of radium emanations applied upon the long bones in two cases of chronic myelogenous leucemia has been studied.

2. The mechanism of action of radium upon the leucemic foci seems to be a complex one as if it is of a generalized as well as localized nature, therefore presupposing the intervention of some intermediate means of action, probably leucotoxins, liberated from leucocytes directly affected by radium, or the carrying of the radium emanation on the blood stream.

3. Radium acted first in these cases, especially if applied in small doses, as a stimulant, on both leucocytes and erythrocytes. On the erythrocytic series, the stimulating effect was especially evident on the cells of the embryonic type (megaloblasts).

4. Following the period of stimulation, radium manifested its destructive action especially on the leucocytes, the order of their disappearing being in direct proportion to their immaturity. Platelets were also greatly reduced by radium, and the coagulation time was proportionately lengthened.

5. There was a *"free interval"* following the radium treatment during which the morphology of the blood remained near normal, and the clinical symptoms of the disease were quiescent.

6. In their reappearance in the blood stream, the immature cells followed exactly the opposite order of their disappearance, the most immature forms being the last to reappear.

7. Hemocytoblasts, or primordial cells, readily disappeared from the blood. Myeloblasts and promyelocytes, next to disappear, acted in a similar manner, confirming their close morphologic relationship. Myelocytes and metamyelocytes disappeared very slowly, acting more like mature cells. Prolymphocytes and lymphoblasts, usually considered as immature forms of lymphocytes, acted more or less like mature cells under radium.

8. Evidence has not been found of circulating lymphocytes being more affected by radium than the granulocytes. In fact, like the majority of investigators, we have found that, while extensive radiation reduces the absolute number of both types of cells, the relative percentage of lymphocytes is usually increased.

REFERENCES

[1]Renon, L., Degrais, Dreyfus: Comp. Rend. de l'Acad. de Med., June 17, 1913.
    Congrès de Londres, August, 1913.
    Desbouis: Bull. et Mem. Soc. Med. des Hop. de Paris, 36:54, (July 19) 1913.
    Bull. et Mem. Soc. Med. des Hop. de Paris, Nov. 28, 1913, xxxvi, 649.
[2]Schueller, H.: Berl. klin. Wchnschr., February 10, 1914, vii, 293.
[3]Gulland, G. Lowell: Brit. Med. Jour., August 20, 1921, ii, 271.
[4]Ordway, T.: Boston Med. and Surg. Jour., April 5, 1917, clxxvi, 14:490.
    Trans. Assn. Am. Phys., 31:177, 1916.
[5]Falta, W., Kriser, A., Zehner: München. med. Wchnschr., 1912, lix, 734.

6Kauffman, M.:   Therap. Monatschr., 1915, xxix, 324.
7Pappenheim, A.:   Med. Klin., 1908, iv, 1244.
7(a) Goodpasture, E. W.: Jour. Lab. and Clin. Med., 1919, iv, 442.
8Ferrata, A.:   Le Emopatie, vol. 1; Soc. Edit. Libraria, Milano, 1918.
9Whitcher, B. R.:   Boston Med. and Surg. Jour., Sept. 7, 1922, clxxxvii, 10.
10Jaksch, V. R.:   Prager med. Wchnschr., 1890, xv, 387, 403, 414.
11Giffin, H. Z.:   Boston Med. and Surg. Jour., Nov. 15, 1917, clxxvii, 686.
12Peabody, F. W.:   Boston Med. and Surg. Jour., Dec. 20, 1917, clxxvii, 873.
13Schisler, E., Brown, E. E.:   Med. Rec., March 26, 1921, xiii, 505.
14Oppenheimer: Berl. klin. Wchnschr. Nov. 14, 1921, lviii, 1351.
15Renon, Degrais, Thibaut: Comp. Rendus Soc. de Biologie, May 3, 1913, p. 937.
16Giffin, H. Z.:   Med. Rec., 1918, xciv, 168, 1020.
17Neu, O.:   München. med. Wchnschr., Nov. 11, 1921, lxviii, 1452.
18Lazarus-Barlow, W. S.:   Medical Research Council, Special report Series No. 62:33;
        London, His Majesty's stationery office, 1922.
19Price-Jones, C.:   Ibid., p. 141.
20Mottram, J. C.:   Arch. of Rad. and Electr., December, 1920, xxv, 197.
21Heinecke, H.:   Mitth. a. d. Grenzgeb. d. Med. u. Chir., 1905, xiv, 21.
22Murphy, J. B., and Ellis, A.W.M.: Jour. Exp. Med., 1914, xx, 397.
23Mottram, J. C.:   Arch. of Rad. and Electr., December, 1920, xxv, 197.
24Mottram, J. C., and Russ, S.:   Jour. Exp. Med., 1921, xxxiv, 271.
25Russ, S.:   Brit. Med. Jour., August 20, 1921, ii, 268.
26Russ, S., Chambers, H., and Scott, G.:   Arch. of Rad. and Electr., May 1921, xxv, 377.
27Mottram, J. C.:   Brit. Med. Jour., August 20, 1921, ii, 269.
28Warthin, A. S.:   Internat. Clinic., Feb. 15, 1906, iv, 243.
29Leitch, A.:   Arch. Rad. and Electr., September, 1921, xxvi, 122.
30Bordier, H.:   Bull. et Mem. Soc. de Radiol. Méd. de France, 1921, ix, 158.
31Renon, L., Degrais and Tournemelle: Bull. et Mem. Soc. med. des Hop. de Paris, Mar.
        20, 1914, xxxvii, 526.
32Murphy, J. B., and Morton, J. J.:   Jour. Exp. Med., Aug. 1, 1915, xxii, 204.

STUDIES IN BLOOD CELL MORPHOLOGY AND FUNCTION*

No. III—Extreme Neutrophilic Leucocytosis with a Note on a Simplified Arneth Count

By Carlos Pons, M.D., and E. B. Krumbhaar, M.D., Ph.D., Philadelphia, Pa.

EXTREMELY high leucocyte counts (over 60 or 80,000 per c.mm.) are almost always found in clinical medicine to be due to some disease of the blood-making organs, such as leucemia. When rarely found to be due to other causes, they are at least accompanied by the appearance in the peripheral blood of a considerable number of immature forms, such as myelocytes and lymphoblasts, so that a more or less accurate "leucemoid" picture is produced. In neutrophilic leucocytosis. on the other hand, as is well known, the white cell count rarely exceeds 40 to 50,000. Such blood pictures are found in a variety of conditions, such as the various acute infectious, toxic conditions, tumor and after severe hemorrhage. It is perhaps not so well recognized that in these conditions, the proportion of young neutrophiles is particularly increased, and that the combination of hemorrhage and ulcerating cancer are especially potent factors in producing such a leucocytosis, and finally that these very high counts may be produced by a summation of stimuli more easily than by a single factor.

The following case is reported as an example of neutrophilic leucocytosis, greater in magnitude than we have been able to find in the literature, and due to an ulcerated, bleeding cancer, with hemorrhage as the chief factor.

CASE: C. S., American, white, female, aged sixty-one, was admitted to the service of Drs. Carnett and Jeffries at the Philadelphia General Hospital on July 8, 1923, with an ulcerated, bleeding breast.

The present illness began two years before, following an injury to the left breast. Three weeks later a lump appeared under the nipple, which gradually grew larger. The left breast had been removed for carcinoma at the Lankenau Hospital, December, 1922. A second operation was performed for recurrence at the same hospital, and since then the patient has felt worse, complaining of cough, loss of weight, and hemorrhage from the breast.

TABLE I

BLOOD FINDINGS

DATE	HB.	R.B.C.	W.B.C.	POLYS*	LYMPHO.	MONO.	META-MYELOCYTES	MYELOCYTES
6-11-23	46		44,000	95	4	1	0	0
6-12-23			94,500					
6-13-23			105,000	96	4	0	0	0
6-14-23			106,000	92	2	2	2	2
6-16-23	35	186,000	120,000	92	2	2	0	4

*Unsegmented (young) forms of polymorphonuclear nucleus constantly averaged 82 to 84 per cent of the total leucocyte count.

When admitted to this hospital, she complained of weakness, a craving for water and bleeding from the ulcer in the left breast. When the breast was dressed, the hemorrhages were so profuse as to be alarming. Although there was superficial infection of the ulcer, no signs of sepsis were present. The patient died nine days after admission.

Autopsy.—(Dr. John Eiman, P.G.H. No. 7809). An adult female, weighing 146 pounds. Nutrition very good. The main finding was a large ulcerated cancerous area, beginning at the cartilage of the fifth rib, on the left side, and extending posteriorly to the midline and superiorly from the second rib to the seventh rib anteriorly. The ulcerated area was covered with soft fungous, reddish-yellow tumor masses. Axillary and subclavicular lymphnodes were not enlarged. The left lung showed a metastatic nodule, 4 cm. in diameter. Bone marrow removed from right tibia was dark brick red in color. The other findings were without significance.

Histologic examination confirmed the gross diagnosis of adenocarcinoma and showed an extreme hyperplasia of the bone marrow. Blood culture at autopsy showed B. coli communior.

EXPERIMENTAL WORK

The metastatic nodule in the lung was macerated and injected into rabbits, to try to ascertain whether the tumor had any stimulating properties on the bone marrow with the production of leucocytosis. The results of these injections were entirely negative.

DISCUSSION

In considering the probable factors instrumental in the production of this extreme leucocytosis, the breast cancer was not thought to be solely responsible. The infection also was not considered in itself sufficient to account for the leucocytosis, as it was superficial and accompanied by a negative antemortem blood culture and without constitutional signs of sepsis. Leucocytosis was present several days before the patient was in a moribund condition, so that although this might have affected the last count, it is not in itself sufficient to say that the condition was due to agonal leucocytosis.

The hemorrhages were recurrent and increasingly severe, and it can therefore be inferred that under the stimulating preparation given by the other factors mentioned, the hemopoietic system was able to respond with unusual efficiency to the extra well recognized stimulus of the blood loss.

In regard to the percentages of segmented and nonsegmented nuclei of the polymorphonuclear leucocytes, it has now been our custom for some months to use this subdivision as a simplification of the Arneth[1] formula. Although we believe that this latter represents a true indication of the age of the leucocyte, nevertheless, like many others, we have found it impractical for routine clinical purposes. In the same way the Schilling[2] modification, though a step in the right direction, has also been found too complicated for routine use. We believe that the essential clinical purpose is served if all neutrophiles are subdivided into: 1, metamyelocytes (very young)—with round or slightly indented nuclei; 2, nonsegmented forms (young)—where the nuclear material is connected by broad bands; 3, segmented forms (older)—where two or more groups of nuclear material are joined by narrow filaments. As metamyelocytes, like their immediate ancestors, the myelocytes, are usually segregated in a differential count from the riper cells of the granulocyte

series, this only necessitates the formation of one extra group for routine work. And, as Arneth, Schilling and various other authors have pointed out, information of considerable clinical value can thereby be ascertained, especially in the acute infections. It will be noted that in the present case the number of polymorphonuclear neutrophiles with unsegmented nuclei,
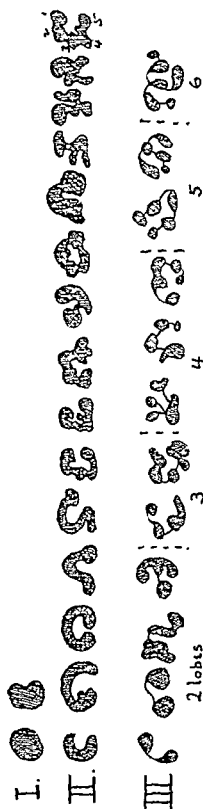
Fig. 1.—Various forms of the neutrophile nucleus, as seen in stained spreads. In the first row are metamyelocytes. In the second row, unsegmented (young) forms. In the third row, segmented (old) forms, with 2 to 6 lobes. The five swellings on the last nucleus of the second row are numbered to show a possible confusion with segmented forms. On account of the thick connecting bands, this is considered an unsegmented young form.

varied from 82 to 84 per cent of the total count, as against the normal of from 4 to 10 per cent. A shift in this direction, though not to such an extreme degree, has regularly been our experience in cases where the polymorpho-nucleosis is of recent occurrence. It is taken to indicate the response of the leucopoietic tissues to the demand for more leucocytes, whereby relatively younger cells emerge in the peripheral circulation. Such a very high per-

centage of unsegmented forms is, of course, almost as unusual as is the total count itself, the usual increase of these forms in the ordinary leucocytosis being to about 30 or 40 per cent.

SUMMARY

1. A case of extreme' leucocytosis occurring in an infected, bleeding carcinoma of the breast is reported (120,000 leucocytes per c.mm.).

2. Several factors were held to be responsible for the leucocytosis: (a) The cancerous growth; (b) Superficial infection; (c) Antemortem leucocytosis; and especially (d) Hemorrhage.

3. A simplification of the Arneth scale is recommended for routine use, as an indication of the age of the leucocyte.

REFERENCES

[1]Arneth, J.: "Die Neutrophilen Weissen Blutkörperchen, etc.," Jena, Fischer, 1904; Zeits. f. klin. Med., 1922, xcv, 201; Zeits. f. Kinderheilk., 1923, xxxiv, 263.
[2]Schilling, V.: Zeits. f. klin. Med., 1920, lxxxix, 1.

STUDIES IN BLOOD CELL MORPHOLOGY AND FUNCTION*

IV. The Arneth Count with Supravital Staining under "Living Blood" Conditions

By C. N. Colbert, M.D., Philadelphia, Pa.

THE recent observations of Sabin[1] on supravital staining of preparations of human blood have suggested an additional field for investigation of the behavior of the leucocytes. The problem of the so-called young neutrophile has been an unsettled one, and it was thought that by the examination of supravitally stained preparations it might be ascertained (1) whether the unsegmented nucleus of the young neutrophile ever becomes segmented during examination; (2) whether this change is reversible; and (3) whether the percentage of unsegmented forms is the same in fixed preparations as in the living ones. These findings would have considerable bearing on the value of the Arneth count or its various modifications. No attempt was made to discriminate between forms having different numbers of nuclear segments, and the division into segmented and unsegmented forms was made on the basis suggested in paper III of this series.

METHODS

The technic as described by Dr. Sabin was used in making preparations. Various vital stains were used at first, but it was found that Grubler's cresyl-blue outlined the nucleus most distinctly, and was therefore adopted as routine. It was found that the stain worked best when the saturated alco-

holic solution was prepared on the same day that the diluted stain was to be used. The life of a preparation in our hands is about three hours, after which time parts of the film become unfit for examination. Janus green, even in small amounts, was too toxic for use.

Blood was obtained from a variety of sources, normal and pathologic. For obtaining large numbers of neutrophiles with a high percentage of unsegmented nuclei, the bloods of a few pneumonia cases showing a high leucocytosis were used. As very thin preparations were necessary definitely to outline the nucleus, the viscosity made considerable difference in the percentage of satisfactory films obtained, and the easiest blood to handle was that of cases of rather marked anemia.

In each preparation, several neutrophiles with unsegmented nuclei were identified, and the outline of each nucleus was repeatedly traced during the life of the film. In no instance did any nucleus, once identified as an unsegmented type, become segmented under observation. No difference in motility or in the nature of the cytoplasm was noted in the two forms.

It was often the case that for some minutes the nature of the nucleus would be in doubt, because of the temporary grouping together of segments and the shortening of the filamentous bands connecting them. When these doubtful nuclei became extended in renewed motion, however, the refractile nuclear segments immediately became distinct. When once these segments were determined in any nucleus, they could be recognized throughout any temporary morphologic changes, and when relieved from pressure, retained their original shape with remarkable constancy. Such a nucleus could not be confused with the unsegmented type.

TABLE I

CLIN. DIAG.	CASE	STAIN	TOTAL LCT. COUNT.	DIFFERENTIAL COUNT IN PERCENTAGES					
				SEGMENTED	UNSEG.	EOSINO.	BASO.	LYMPHO	LARGE & TRANS.
Normal	I	Wright's Vital	9,650	56 55	1 5	2 2	1 0	37 37	3 1
Lobar Pneumonia	II	Vital	36,300	46 41	40 44	1 2	0 0.5	13 11	0 1.5
Lobar Pneumonia	III	Vital	10,200	59 53	12 20	1 0	0 1	25 23	3 3
Lobar Pneumonia	IV	Vital	24,000	80 79	6 11	1.5 1	1 1	9.5 7	2 1
Sec. Anemia	V	Vital	5,600	71 68	3 2	4 3	0 0	21 26	1 1

Differential counts were made on several specimens, grouping all segmented forms together, with the unsegmented forms as a separate class. A check was made by a similar count on a fixed film stained with Wright's stain. Five of these counts are shown in Table I.

In a somewhat greater proportion than in these tables, it was found that the fixed films showed a slightly larger percentage of unsegmented forms than did the vital stain. Some of this difference may be due to the fact that a greater percentage of the unsegmented forms had to be rejected in the

count as not definitely capable of being classified. Undoubtedly some of the horseshoe forms stained with Wright's were actually segmented nuclei in this configuration. Nevertheless the discrepancy is not wide, and the percentage of unsegmented forms is undoubtedly as constant as the percentage of any of the other formed elements of the blood.

COMMENT

Vitally stained living blood films offer an interesting means of corroborating Arneth counts made on stained fixed smears. As the normal life of a leucocyte in the circulation is thought to be only a matter of 2 or 3 days, the constant shape of the neutrophile nucleus in the living state for several hours is further support of the view that this shape is not an accident, but bears a definite relation to the life history of the cell.

A drawback to this method, as Carrel[2] has suggested, is that because of lack of oxygen and fresh media, to remove waste products, the changes are mostly degenerative. The technic could doubtless be modified with this in mind. There are some difficulties in the way of using this method routinely. The percentage of successful preparations is not so great as by polychrome staining. In examining motile neutrophiles, several minutes may be required to determine the nature of each nucleus, making the procedure very lengthy.

SUMMARY

1. Segmentation of the unsegmented neutrophile nucleus was not observed over a period of several hours in preparations of living leucocytes vitally stained.

2. Segmented neutrophile nuclei were not seen to become horseshoe nuclei, although the segments may become temporarily indistinguishable during motion.

3. The relative percentage of segmented and unsegmented nuclei of neutrophiles is approximately the same in living as in fixed preparations.

4. These findings support Arneth's view of the significant relation between the shape of the nucleus and the age of the cell.

REFERENCES

[1]Sabin, F.: Johns Hopkins Hosp., Bull., 1923, lxxxiv, 277.
[2]Personal communication.

THE USE OF DESICCATED RED BONE MARROW AND SPLEEN IN THE TREATMENT OF ANEMIA*

By WILLIAM THALHIMER, M.D., MILWAUKEE, WIS.

LEAKE[1] and his collaborators, at the University of Wisconsin, have recently reported the results of their investigations of the hematopoietic effect of desiccated red bone marrow and spleen, combined in equal proportions by weight (referred to hereafter as "spleen-marrow compound"), in animals, normal human beings, and patients with anemia. A brief summary of their results is as follows. This preparation when administered either intravenously or orally to rabbits, orally to dogs, and orally to normal beings, caused a very rapid and marked increase in the number of circulating erythrocytes, and a somewhat less increase in hemoglobin. Administration of this preparation by mouth to patients with secondary anemia was followed by prompt beneficial results with a lasting relief of the anemia in most patients. Patients with primary anemia were not benefited, but on the contrary this compound caused a rapid fall in hemoglobin and circulating erythrocytes. This therapeutic test in doubtful pernicious anemia cases can be used as a diagnostic aid.

These investigators have suggested that independent clinical studies be made of the value of spleen-marrow compound, and the following report contains the results of my observations of the effect of this agent in patients with secondary and primary anemia.†

A review of the literature can be found in Leake's articles and need not be repeated here.

METHODS, CLINICAL MATERIAL AND RESULTS

All patients who were given capsules of spleen-marrow compound were carefully followed with erythrocyte counts and hemoglobin determinations. The blood counts were made by experienced technicians, using the usual technic, and either the Dare or Sahli hemoglobinometer. Each patient was followed by the same technician, and the technicians were instructed to be careful not to read the hemoglobin determinations too high. The results can be considered as demonstrating the relative increase and decrease in hemoglobin, even though the clinical hemoglobinometers used might not be relied on for absolute hemoglobin values. The erythrocyte counts are to be relied on both for their absolute and relative values.

The reason several technicians furnished the clinical laboratory work

*From the Laboratories of Columbia Hospital.

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†Spleen-marrow compound, already prepared in 5-grain capsules, was secured through the courtesy of Dr. David Klein, of the Wilson Laboratories, Chicago.

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is that the observations were made on patients in several hospitals* and on private patients of a number of physicians. I believe that because the patients were followed in several laboratories, the results are more dependable than if the blood counting had been done in a single laboratory. The results of the different technicians automatically act as checks, and actual checks were obtained on several outside patients from time to time by the technician at Columbia Hospital, the blood counts agreeing within the usual limits of error.

Practically all the patients were given three 5-grain (0.3 gram) capsules of the preparation a day; i.e., one capsule before or after each meal. No side actions were noted. No selection of patients was made.

The table of the results is practically self-explanatory and needs very little amplification. The records are reported as they stand at this time. Some patients have been followed long enough to give final results. Others have been followed only for a short time. The results are arranged in no special order except that grouping has been made of patients with secondary anemia showing improvement, those showing no improvement, and those with pernicious anemia.

Forty-six patients with secondary anemia have received spleen-marrow compound. Forty-one patients (89 per cent) showed an increase in circulating erythrocytes and hemoglobin, and five patients (11 per cent) did not. In many patients an increase of the erythrocytes occurred in from one to three days. The increase in the hemoglobin was usually more tardy and its continued rise more gradual. Some of the patients showed an increase in hemoglobin from an initial value of 60 per cent to a final value of about 90 per cent, and of erythrocytes from about 3,000,000 at the beginning of treatment to about 5,000,000 per cubic millimeter in several weeks.

Two patients (16 and 20) had been given iron and arsenic intravenously with slight or no improvement. Spleen-marrow compound was then given and improvement followed.

One patient (8) who improved after a short term of medication, but before a lasting effect was produced, refused to continue treatment. The blood count and hemoglobin promptly fell.

In a few anemic patients (5, 7, 25) whose blood findings rose very rapidly to normal, or slightly above normal, medication was immediately discontinued. A week later a moderate fall in the blood count was found. Treatment was resumed and was followed by a prompt rise in erythrocytes and hemoglobin. This evidence indicates a direct relationship between administering the extract of red bone marrow and spleen and the increase in the patient's hemoglobin and circulating erythrocytes.

Although in some patients the blood findings increase markedly or even return to normal after only two or three weeks, it seems that treatment must be continued for six or eight weeks for the improvement to become permanent.

TABLE OF RESULTS

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
1	Mitral Stenosis and Regurgitation. Male, age 51.	2-9	2-9	3,790,000	70	
			2-10	3,920,000	75	
			2-11	4,035,000	80	
			2-18	4,410,000	88	
			2-25	4,060,000	87	Patient feels much improved, less dyspnea.
2	Postoperative suppuration of wounds. Male, age 26.	2-9	2-9	3,170,000	60	
			2-10	3,750,000	70	
			2-11	4,360,000	78	
			2-13	4,235,000	82	
			2-18	4,280,000	80	
3	Postoperative suppuration of wounds. Male, age 32.	2-9	2-9	2,720,000	50	
			2-10	3,300,000	58	
			2-11	3,220,000	61	
			2-13	3,290,000	63	
			2-19	3,570,000	70	
			2-25	3,480,000	73	
			3-3	3,160,000	75	
4	Chronic Pleurisy with effusion. Male, age 21.	2-9	2-9	2,880,000	50	
			2-10	3,220,000	55	
			2-11	3,220,000	60	
			2-13	3,710,000	72	
			2-18	3,820,000	71	
			2-25	4,810,000	88	
5	Menorrhagia. Age 39.	2-10	2-10	3,160,000	60	
			2-13	4,080,000	72	
			2-15	5,580,000	88	Medication discontinued.
			2-20	4,720,000	79	Medication resumed.
			2-25	5,200,000	91	
6	Lung abscess	2-12	2-12	3,780,000	65	
			2-13	3,232,000	66	
			2-18	3,400,000	65	
			2-20	4,080,000	70	
			2-22	4,101,000	70	Medication discontinued.
			3-10			Medication resumed.
			3-18	4,180,000	76	
			3-21	5,320,000	84	
7	Bilateral Pyonephrosis. Male, age 33.	2-13	2-13	3,490,000	63	
			2-14	3,070,000	60	
			2-15	3,310,000	64	
			2-18	4,210,000	84	Medication discontinued.
			2-25	3,720,000	68	Medication resumed.
			2-29	4,320,000	84	

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
8	Diagnosis undetermined. Male, age 68.	2-15	2-15	3,550,000	70	Patient refused to take capsules after this.
			2-18	4,410,000	86	
			2-25	3,610,000	68	
			3-3	2,860,000	64	
9	Suppurating tuberculous hip. Male, age 25.	2-16	2-16	3,540,000	62	Patient refused to take capsules because he was suffering from abdominal cramps.
			2-17	3,660,000	57	
			2-20	3,385,000	65	
10	Polyarticular Arthritis. Male, age 29.	2-20	2-20	3,530,000	70	
			2-22	3,480,000	74	
			2-27	4,100,000	80	
			3-3	4,390,000	86	
11	Hemolytic Icterus splenectomy, 11 mos. ago. Female, age 24.	1-22	1-22	3,580,000	71	
			1-29	3,580,000	76	
			2-13	3,750,000	73	
			2-18	3,910,000	75	
			2-26	4,110,000	79	
			3-11	4,160,000	78	
12	Secondary anemia, cause not discovered. Female, age 18.	2-14	2-14	3,260,000	49	
			3-5	3,520,000	53	
			3-14	4,010,000	58	
13	Secondary anemia, cause not discovered. Female, age 27.	2-4	2-4	3,700,000	79	
			2-23	4,340,000	83	
			3-2	4,320,000	84	
14	Secondary anemia, cause not discovered. Female, age 32.	1-30	1-30	4,250,000	81	
			2-7	4,400,000	83	
			2-13	3,920,000	83	
			2-20	4,290,000	87	
15	Secondary anemia, cause not discovered. Female, age 36.	1-8	1-8	4,070,000	76	
			1-15	4,310,000	76	
			1-23	4,020,000	80	
			1-30	4,010,000	77	
			2-13	4,340,000	80	
			2-20	4,200,000	85	
			3-5	4,350,000	84	
			3-14	4,380,000	86	
16	Secondary anemia, cause not discovered. Female, age 22.	1-1	1-1	4,000,000	57	Previous to taking this preparation the patient had three intravenous injections of iron and arsenic with no relief of the anemia.
			1-5	4,270,000	64	
			1-12	4,440,000	69	
			1-19	4,280,000	70	
			1-27	4,280,000	70	
			2-2	4,440,000	76	

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
17	Secondary anemia, cause not discovered. Female, age 25.	2-18	2-18 2-28 3-6	3,110,000 3,850,000 4,070,000	77 78 82	
18	Secondary anemia, cause not discovered. Female, age 30.	1-28	1-28 2-8	3,520,000 3,760,000	74 79	
19	Secondary anemia, cause not discovered. Female, age 38.	11-22-23	11-22 12-6 12-13 1-5	3,230,000 4,360,000 4,990,000 5,030,000	60 71 83 90	
20	Secondary anemia, infantile uterus, menorrhagia, menstrual blood loss less than normal.	11-19-23	11-19 12-3	4,010,000 4,670,000	84 96	Oct. 15, 1923 the hemoglobin was 73% and after two intravenous injections weekly of iron and arsenic for five weeks the hemoglobin on Nov. 19, 1923, was 84%.
21	Secondary anemia menorrhagia, age 27.	11-21-23	11-21 12-6 1-8	4,080,000 4,010,000 4,450,000	76 84 88	
22	Secondary anemia cause not discovered. Female, age 44.	10-19-23	10-19 11-16	4,030,000 4,200,000	43 51	Shape of erythrocytes suggestive of pernicious anemia.
23	Secondary anemia Pulmonary tuberculosis suspect. Female age 24.	11-22-23	11-22 12-10 1-27	4,030,000 4,430,000 4,650,000	78 84 91	
24	Secondary anemia cause not discovered. Female, age 18.	2-23	2-23 3-5 3-11	4,640,000 4,040,000 4,940,000	70 69 71	
25	Ruptured Ectopic pregnancy. Age 33.	12-18-23	12-3 12-18	3,990,000 3,630,000	41 50	Operation Dec. 10. Diagnosis verified. Right tube and ovary removed and as much as possible of intraperitoneal blood clot was removed.

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINIS- TERED	DATE	ERYTHROCYTES	HEMO- GLOBIN PER CENT	REMARKS
25 Cont'd			12-20	4,200,000	67	
			12-22	4,530,000	68	
			12-24	4,370,000	68	
			12-26	4,000,000	71	Patient went home but continued medication.
			1-15	4,720,000	80	
			2-15	5,710,000	95	Discontinued medication.
			3-4	4,580,000	83	Resumed medication.
26	Secondary anemia, acute otitis media. Female, age 40.	2-7	2-7	4,260,000	78	
			2-18	4,250,000	79	
			3-4	4,650,000	82	
27	Slight anemia, patient felt weak and looked paler than her blood count would indicate. Female, age 23.	2-8	2-8	4,540,000	90	
			2-15	5,100,000	95	Medication discontinued.
28	Secondary anemia, cause not discovered.	2-15	2-15	3,450,000		
			2-21	3,720,000	75	
			2-26	3,870,000	80	
			2-28	3,920,000	80	
			3-11	3,920,000	78	
29	Secondary anemia, cause not discovered. Male, age 28.	3-1	3-1	3,500,000	68	
			3-10	4,720,000	100	
30	Active pulmonary tuberculosis. Male, age 28.	2-23	2-23	4,800,000	90	
			2-25	5,200,000	90	
31	Active pulmonary tuberculosis. Male, age 30.	2-23	2-23	4,400,000	85	
			2-27	4,920,000	91	Medication discontinued.
			3-12	4,290,000	78	Medication resumed.
			3-18	4,830,000	88	
			3-21	4,288,000	89	
			3-25	4,520,000	87	
32	Active pulmonary tuberculosis. Male, age 26.	2-23	2-23	4,288,000	85	
			2-27	4,736,000	88	
33	Active pulmonary tuberculosis. Male, age 27.	2-23	2-23	4,168,000	90	
			2-26	5,180,000	100	
34	Postoperative infection, (cesarean section.) Jan. 5. Age, 33.	2-7	1-12	4,850,000	88	
			1-18	3,910,000	60	
			2-7	3,480,000	53	
			2-11	3,900,000	51	
			2-13	3,900,000	55	Patient left hospital, no further observations.

TABLE OF RESULTS (CONT'D)

PATIENT	DIAGNOSIS	FIRST DATE ADMINIS-TERED	DATE	ERYTHROCYTES	HEMO-GLOBIN PER CENT	REMARKS
35	Active pulmonary tuberculosis. Male, age 34.		3-3	4,190,000	90	
			3-12	4,830,000	94	
			3-18	5,090,000	95	
			3-21	5,160,000	95	
			3-25	5,000,000	96	
36	Secondary anemia, cause not discovered. Female, age 30.	3-14	3-14	3,590,000	60	
			3-15	4,040,000	60	
			3-16	4,320,000	62	
			3-17	4,360,000	65	
37	Postoperative rectal hemorrhage, (Oper. for prolapse of rectum). Male, age 29.	3-20	3-20	2,340,000	45	
			3-23	3,010,000	60	
38	Fibromyoma of uterus and bleeding hemorrhoids Age 38.	3-12	3-12			
			3-15	3,980,000 4,590,000	75 80	Medication discontinued. Hysterectomy, performed Mar. 18. Medication resumed.
			3-21	3,560,000	64	
			3-23	4,080,000	77	
39	Von Jaksch anemia. Male, age 2.	3-16	3-14	1,600,000	45	W. B. C. 25,000 no abnormal leucocytes, but 7 normoblasts per 100 leucocytes.
			3-17	1,570,000	47	W. B. C. 15,000; 4 normoblasts.
			3-18	2,000,000	48	W. B. C. 13,000; 3 normoblasts.
			3-19	2,060,000	48	W. B. C. 10,200; no normoblasts.
			3-20	2,060,000	53	W. B. C. 10,000; no normoblasts.
			3-21	2,640,000	53	W. B. C. 10,400; no normoblasts.
			3-22	2,500,000	54	W. B. C. 10,000; no normoblasts.
			3-23	2,630,000	54	W. B. C. 10,200; no normoblasts.
			3-24	2,860,000	60	W. B. C. 11,000; no normoblasts.
			3-25	2,900,000	60	W. B. C. 10,000; no normoblasts.
			4-1	3,900,000	70	
40	Mild purpura-occasional small skin hemorrhages. Female, age 34.	2-15	2-19	4,000,000	75	
			2-29		80	
			3-13	4,300,000	83	
41	Secondary anemia, cause not discovered. Female, age 22.	12-10 23	12-10	3,900,000	78	
			12-21	4,130,000	82	
			12-29	4,150,000	86	
			2-1	4,000,000	85	Menstruating at present.

Patients with Secondary Anemia Showing no Improvement

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
42	Multiple abdominal sinuses after multiple intra-abdominal abscesses following acute appendicitis.	11-5-23	11-5	4,740,000	82	
			11-8	4,650,000	85	
			11-16	5,000,000	85	
			11-26	5,250,000	86	
			12-1	4,750,000	84	
			12-8	4,690,000	82	
			12-18	4,750,000	84	
43	Six months' pregnancy. No reason found for anemia. Age, 24.	2-8	2-8	3,970,000	63	
			2-27	3,600,000	57	
			3-5	3,630,000	42	Medication stopped.
44	Child, male, age 3 yrs., history of intestinal worms. Stool examination negative.	2-19	2-19	3,920,000	52	
			2-26	3,950,000	53	
			3-10	3,780,000	42	
45	Secondary anemia, Female, age 70 yrs. Malignancy of stomach suspected.	2-26	2-26	4,560,000	88	
			3-5	4,500,000	86	
			3-11	4,460,000	82	
46	Active pulmonary tuberculosis.	2-24	2-24	4,288,000	85	
			2-26	4,160,000	88	
			2-27	4,730,000	88	
			3-12	4,560,000	84	
			3-18	4,290,000	86	
			3-21	4,180,000	87	
			3-24	4,110,000	86	

Patients with Pernicious Anemia

PATIENT	DIAGNOSIS	FIRST DATE ADMINISTERED	DATE	ERYTHROCYTES	HEMOGLOBIN PER CENT	REMARKS
47	Pernicious Anemia		1-22	2,500,000	62	
		1-23	1-25	1,250,000	45	Medication discontinued.
			2-19	1,800,000	58	
48	Pernicious Anemia	2-16	2-16	2,020,000	65	Diagnosis not clear before medication.
			2-19			
				1,200,000	43	Medication discontinued.
49	Pernicious Anemia	2-18	2-18	1,400,000	30	
			2-19	1,232,000	29	
			2-20	992,000	30	Medication discontinued.
50	Pernicious Anemia	1-2	1-2	3,610,000	67	
			1-5	2,980,000	60	
			1-7	2,520,000	52	Medication discontinued.

In four patients with pernicious anemia, administration of the capsules caused a prompt decrease in both hemoglobin and circulating erythrocytes. Therefore, this preparation should not be used in this condition. However, one patient with Von Jaksch's anemia (39), has shown a marked improvement in both clinical condition and blood picture during ten days of treatment. The red cell count increased from 1,600,000 to 2,900,000 per cubic millimeter, and the hemoglobin from 45 per cent to 60 per cent. The leucocyte count decreased from 25,000 to 10,000 per cubic millimeter, and normoblasts which were present before treatment in the ratio of seven to every one hundred leucocytes, have not been present since the third day after spleen and bone-marrow medication was begun.

It is interesting that in one patient with hemolytic icterus, whose spleen had been removed eleven months previously, hematopoiesis was stimulated by the preparation.

The satisfactory response of the patients with pulmonary tuberculosis is also of interest, and may be of considerable importance.

In all patients, subjective improvement accompanied the relief of the anemia. It must be remembered that vigilance should not be relaxed in attempting to determine the cause of the anemia, even though improvement follows red bone marrow and spleen therapy in some cases of secondary anemia which have an obscure etiology.

SUMMARY

The administration of desiccated spleen and red bone marrow, combined in equal proportions, in three five-grain capsules daily, to a group of forty-six patients with secondary anemia was followed by a definite increase in hemoglobin and circulating erythrocytes in 89 per cent of these patients.

This preparation caused a fall in hemoglobin and circulating erythrocytes in four patients with pernicious anemia, and therefore, should not be used in this condition.

These results are confirmatory of the observations of Leake and his collaborators.

REFERENCES

1 Leake, Chauncey D., and Leake, Elizabeth W: the Erythropoietic Action of Red Bone Marrow and Splenic Extracts, Jour. Pharmacol. and Exper. Therap., September, 1923, xxii, No. 2, p. 75.

Leake, Chauncey D.: Leucocytic Reactions to Red Bone Marrow and Spleen Extracts, Jour. Pharmacol, and Exper. Therap., September, 1923, xxii, No. 2, p. 109.

Leake, Chauncey D.: The Hematopoietic Effects of Desiccated Red Bone Marrow and Spleen in Normal Humans, Jour. Pharmacol. and Exper. Therap., December, 1923, xxii, No. 5, p. 401.

Leake, Chauncey D., and Evans, J S.: A Preliminary Note on the Use of Red Bone Marrow and Spleen in the Treatment of Anemia, Wisconsin Med. Jour., December, 1923, xxii, No. 7, p. 294.

THE REMOVAL OF PHENOLTETRACHLORPHTHALEIN FROM THE CIRCULATION*

BY R. OTTENBERG, M.D., S. ROSEN, M.D., AND L. GOLDSMITH, M.D.,
NEW YORK CITY

PHENOLTETRACHLORPHTHALEIN, when injected into the blood, is excreted by the liver. Rosenthal's method of testing liver function consists of injecting the dye and testing the blood after fifteen and sixty minutes to see how much of the dye has been removed. We have used this method in more than 100 cases.

Some interesting problems are brought up by observations on the percentage of dye in the blood during the first fifteen minutes after intravenous injection. In fourteen cases a needle was inserted in a vein of the opposite arm and samples of blood were obtained at intervals varying from one, two, three, five or ten minutes after completion of the injection. The data are given in the accompanying chart. In all these cases the amount of dye injected was 5 mg. per kilo of body weight.

The most interesting point is the great speed with which initial removal of the dye from the circulation takes place. Whether there was ultimately dye retention or not, the percentage of dye was reduced from the theoretical 100 per cent which would have been present if all injected were in the circulation, to 20 per cent or less, within the first five minutes. In fact in all the cases in which a two or three minute observation was made it was found that three-fourths of the dye had already been removed from the blood.

Of course, some irregularities are due to the fact that it probably takes several minutes for the dye to become uniformly distributed in the circulation. Also it must be remembered that removal of the dye begins at the beginning of the injection, and the giving of the injection consumes from one to five minutes.

We can understand the rapid removal of the dye when we remember that in volume of blood supply the liver stands first of all the organs of the body. In a dog of 14,300 gm. weight, with a liver weighing 454 gm., Burton Opitz† found that the liver received 422 c.c. of blood per minute, or roughly half the blood in the body. At this rate, assuming perfect mixing, 75 per cent of the body blood should pass through the liver in two minutes and 96 per cent in five minutes.

This initial rapid removal of dye takes place, whether the liver is able to excrete dye or not. In the chart it will be seen that (with the exception of one case in which no reading was made between three and fifteen minutes) the initial drop occurred in all the cases in which the one hour blood showed

10 per cent or more of dye, i.e., cases in which the liver was unable to excrete the dye. The initial removal therefore does not depend on excretion by the liver, but on absorption from the blood. The percentage of dye in the circulation after fifteen minutes represents an equilibrium between the pressure of dye in the blood and the absorbing capacity of the tissues. This point of balance is, of course, lower in the instances in which the liver has already been able to excrete some dye than in those in which the liver is obstructed. The mechanism of this removal requires study; at present it cannot even be stated whether it is by the reticuloendothelial (Kupfer) cells or not, and if by reticuloendothelial cells whether exclusively by those of the liver or also by others.
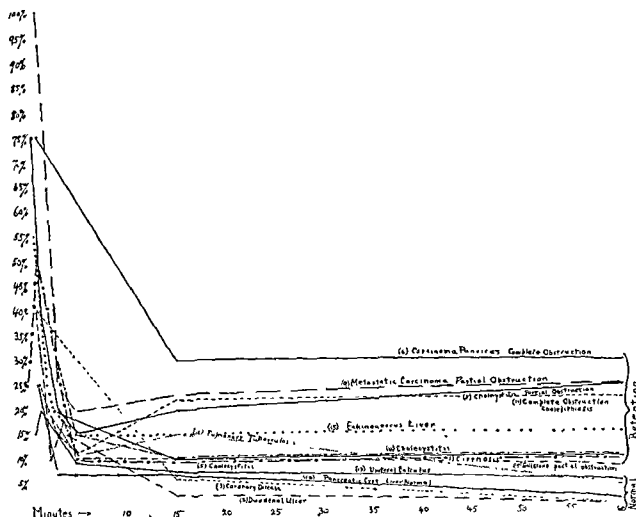
Chart 1.

This offers a partial explanation of the fact that in cases in which the liver is completely obstructed or is badly damaged the percentage of dye in the blood is usually the same at one hour as at fifteen minutes (twenty-three of our 102 miscellaneous cases). It does not, however, explain those cases (nine in number) in which the one hour concentration of dye was higher than the fifteen minute. When we first encountered this, we thought it might be an error, but subsequent observations convinced us it was a reality. We can offer no satisfactory explanation of it, though possibly it may be due to failure of the dye to become uniformly distributed throughout the circulation even in fifteen minutes.

That the body has resources for handling the dye other than excretion by the liver is evident from: (1) the fact that nobody has been able to

account for excretion of more than 75 per cent of the injected dye; (2) in cases of total biliary obstruction the dye disappears from the circulation in twenty-four to forty-eight hours. One of these resources is excretion by the kidneys. This occurs, as shown by Whipple, Peigtal and Clark, only when the liver is badly damaged. We can corroborate this from experience with clinical cases. It is doubtful, however, whether the amount excreted by the kidneys can account for more than a small part of the dye injected.

LESIONS IN GUINEA PIGS PRODUCED BY INOCULATION OF VERY OLD CULTURE OF TUBERCLE BACILLUS*

By Randle C. Rosenberger, M.D., Philadelphia, Pa.

My reason for making the following observations upon an old culture of the human tubercle bacillus, was to see whether it would eventually bring about death of an inoculated animal. In previous experiments, the period of observations had been only six to twelve weeks. With small doses of culture only very small lesions would devleop, and often no perceptible lesions were observed at autopsy.

The culture used in the experiments was originally isolated by Trudeau at Saranac Lake, in 1891, directly from a child's lung and called "R_1" bacillus tuberculosis hom. Baldwin has stated: "It has passed through one rabbit, and since grown on glycerin sheep serum, glycerin agar and glycerin broth successively. During the last ten years it has been grown upon broth containing 5 per cent glycerin and reaching slightly acid; a new transfer was made nearly every month. Its virulence for rabbits and guinea pigs was pronounced at first, but the present culture barely infects guinea pigs even in large doses and then only very slight localized or chronic disease results."

I received this culture in July, 1904, and since that time have kept it growing upon 6 per cent glycerin agar, making subcultures every two or three months during this interval of twenty years. As the organism was originally isolated in 1891, at the time of this experiment it had been under cultivation for thirty-three years.

Two pigs were inoculated intraperitoneally with 2.5 c.c. of a salt suspension of a glycerin agar growth of the strain "R_1" tubercle bacillus, on November 8, 1921.

May 11, 1922, or six months after inoculation, one pig was sacrificed and no appreciable emaciation was noticed.

Upon opening the abdominal cavity a large hour-glass shaped mass 7.5 cm. in length and 2 cm. in thickness and distinctly caseous was found. In addition, small nodules or enlarged glands were observed varying from 0.5 cm. to 1.5 cm. in diameter beneath the diaphragm and one nodule was in the

diaphragm. One small mass was in the lower border of the right lobe of the liver; one nodule, 1 cm. in diameter, was in the tail of the spleen; one in the epididymis; the left testicle was caseous and a globular mass, 2.5 cm. in diameter, (caseous) was adherent to the posterior wall of the abdominal wall on the right side. No microscopic lesions were present in the lungs or kidneys.

Spreads made from all of these nodules contained tubercle bacilli and a few were found in the urine obtained from a greatly distended bladder.

Inoculations were made upon blood serum and glycerin agar, but no growth occurred up to four weeks' time.
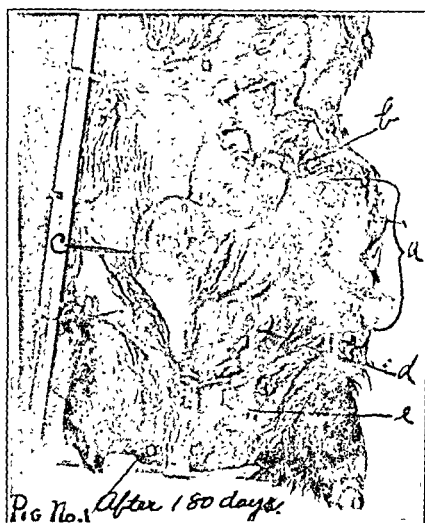
Fig. 1.—Pig No 1, after 180 days. *A*, Mass in abdomen; *B*, nodule upon tail of spleen; *C*, mass adherent to abdominal wall, *D*, lesion in pelvis anterior to rectum; *E*, caseous testicle.

The second pig was allowed to live and the animal was found dead in its cage October 13, 1923.

During the life of the animal, more especially after it had survived the inoculation for about a year, it was noticed that upon a number of occasions it would sit very quietly, objected to being handled, its hair was ruffled and upon examination a small mass was appreciable by palpation in the abdomen.

It would move about with great deliberation, and its bodily movements were not those of a normal animal. During the last few months of its life it appeared listless, would seek the corner of its cage and remain very quiet.

Emaciation gradually set in and death occurred within slightly less than two years' time.

At autopsy, a large mass almost globular, 6 cm. in diameter was found in the abdominal cavity, distinctly caseous, and with adhesions to the large and small intestines. In addition to this large mass, a smaller one was found (also caseous) between the bladder and rectum, and a caseous lesion was adherent to the diaphragm and superior surface of the liver. Firm adhesions were noticed at the base of both lungs, though no gross lesions were present in these organs.

There were a number of adhesions present at the tail of the spleen but no gross lesions were found in this organ, or in the kidneys or liver.
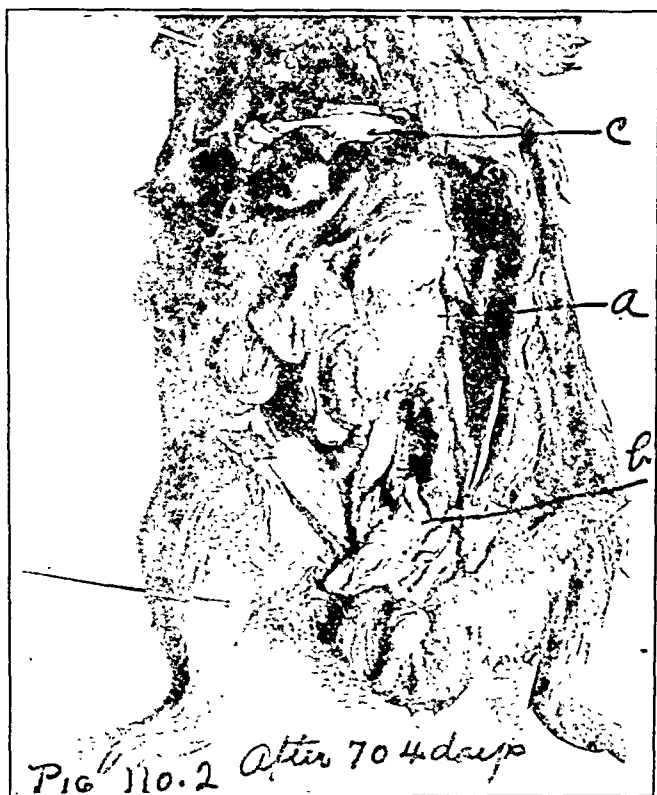
Fig. 2.—Pig No. 2, after 704 days. *A,* Large caseous mass adherent to stomach and intestines; *B,* caseous mass between bladder and rectum; *C,* masses between liver and diaphragm.

Spreads made from all the caseous lesions showed tubercle bacilli. Inoculations were made from the caseous mass in the abdominal cavity into glycerin bouillon but no increase in the number of organisms was observed up to six weeks.

Histologic examination was made of the kidneys, lungs, liver and spleen, but no distinct lesions of tuberculosis were found. The lungs showed a markedly cellular pneumonia, but no tubercles.

This strain of tubercle bacillus, after thirty-three years of cultivation, was capable of producing massive lesions in guinea pigs and ultimately caused the death of the animal studied.

LABORATORY METHODS

A MODIFICATION OF THE BLOOR METHOD FOR BLOOD PHOSPHATES[*]

BY K. LUCILLE McCLUSKEY, PH.D., CHICAGO, ILL.

BRIGGS'[1] modification of the Bell and Doisy method for the determination of inorganic and acid soluble phosphoric acid in the blood appears to be more desirable than previous ones due to its comparative simplicity and equal accuracy.

For a study on the distribution of phosphorus in the cell and plasma of tuberculous blood, it occurred that possibly an extension of the above method to the determination of total and lecithin phosphorus might be advisable. This has been accomplished by a combination of the Briggs' method with that of Bloors.[2]

It was found that a phosphate content ranging from 0.09 mg. to 0.18 mg. of H_3PO_4 when reduced with hydroquinone in acid sulphite solution gave a color of desirable intensity for measurement in a volume of 15 c.c. to 20 c.c. This amount of phosphate corresponds to that found in the samples used for analysis in the Bloor method. For this reason the quantities used here for the analyses have been the same. The method of Bloor has been followed for the preparation and digestion of the blood solutions and should be consulted for details.

Total phosphorus, lecithin, acid soluble and inorganic phosphorus were determined upon whole blood and plasma. The acid soluble and inorganic phosphorus determinations were made upon filtrates obtained by deproteinizing whole blood in some cases with acid ammonium sulphate and in others with trichloracetic acid.

A detailed account of the technic used, is given for the total phosphates. This procedure was followed exactly in all the determinations with the exception of such modifications as are indicated below.

METHOD

Total Phosphates.—To 3 c.c. of whole blood in a 25 c.c. volumetric flask, water is added to the mark and the solution is well mixed. One c.c. of this solution is measured into a large tube (25 mm. x 250 mm.) graduated at 15 c.c. and digestion is carried out over a microburner after 1.5 c.c. of a mixture of equal parts of concentrated sulphuric and nitric acids and two glass beads have been added. After digestion is complete the solution is cooled, 5 c.c. of water added, and the acid titrated with approximately 40

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per cent NaOH (from sodium), using phenolphthalein as indicator. The solution is brought back to the slightly acid side by adding an approximately N/1 H_2SO_4 drop by drop until the color just disappears.

Standard Phosphate Solutions.—To each of two digestion tubes are added an amount of alkali equal to that used in the neutralization of the digestion mixture above and one drop of phenolphthalein. The solution is made acid with dilute sulphuric acid, then just alkaline with one or two drops of sodium hydroxide and brought to the neutral point with the N/1 H_2SO_4. The tubes are cooled, and to one are added 4 c.c. and to the other 5 c.c. of standard phosphate solution, containing respectively the equivalent of 0.12 mg. and 0.15 mg. of H_3PO_4. Then to both the unknown and the standards are added 2 c.c. of Na_2SO_3, 1 c.c. of hydroquinone and the tubes made up to the 15 c.c. mark with water. After the addition of 2 c.c. of ammonium molybdate solution, the tube is at once stoppered tightly with a good cork stopper, well mixed, allowed to stand two to three hours, and read in the colorimeter.

Colorimeter Reading.—The 5 c.c. standard is placed in the colorimeter on the left and the 4 c.c. standard on the right and set at 30.

The standards are considered to be proportional to their phosphate content, if the average of several readings of the 5 c.c. standard lies between 23.7 and 24.3. The unknown is then matched with the standard having the nearer color intensity. Measurements were made in the Kober colorimeter with the standard set at 30.

NOTES ON METHODS

The standards for lecithin and inorganic phosphorus should contain the equivalent of .09 mg. and .12 mg. of H_3PO_4.

The acid soluble phosphorus determinations require a standard of .15 mg. or .18 mg. of H_3PO_4 for whole blood and .15 mg. of H_3PO_4 for plasma.

The standard for the inorganic phosphorus determination made on the acid ammonium sulphate filtrate is made to contain 8 c.c. of acid ammonium sulphate and a period from fourteen to seventeen hours should be allowed for reduction.

To balance the salt content, 1.5 c.c. of acid ammonium sulphate is added to the standard for the acid soluble phosphorus determination of this same filtrate, and the period of reduction is six hours.

The average acidity found by titrating 10 c.c. portions of the filtrates from the trichloracetic acid precipitation corresponds to .75 c.c. of the trichloracetic acid solution. The standards for the inorganic phosphorus are made to contain this amount.

It was observed that when the tubes were left exposed to the air, during the period of reduction, a deep blue layer formed at the surface and gradually spread throughout the tube. This oxidation is prevented by the presence of an atmosphere of sulphur dioxide. For this reason a large quantity of sodium sulphite solution has been used, and the tubes stoppered tightly during the reduction.

Since the temperature affects the speed of reduction of the phosphomolybdic acid, it is necessary before the reagents are added that a uniform

temperature exist. As a matter of routine, the tubes were cooled under the tap to room temperature after neutralization or were allowed to stand until such temperature was attained. During the neutralization process on account of the heat developed and the strong acid solution, the phenolphthalein was not added until most of the acid had been neutralized and the tube cooled.

It was observed that if the molybdate reagent was added in presence of ammonium sulphate and the tubes allowed to stand as short a time as two minutes before the other reagents were added, a faint yellow color became visible and upon the addition of the sulphite and hydroquinone, a greenish-blue color developed immediately which deepened to a dark blue within a short time and was not proportional to the phosphate content. If the reagents are added in the order of sulphite, hydroquinone, and molybdate, this difficulty is eliminated and although the color development is slower in presence of the ammonium salts, it is strictly proportional to the phosphate content. This order of adding reagents has been adopted throughout.

The digestion tubes were graduated at 15 c.c.

Should the tubes become alkaline after the addition of the sodium sulphite, they are made just acid with $N/1$ H_2SO_4 before the ammonium molybdate solution is added.

To ascertain whether the color intensity was still proportional to the phosphate content within the time limits allowed for reduction, the following experiments were tried.

1. One and five-tenths c.c. of sodium hydroxide were measured into each of two tubes and neutralized with dilute sulphuric acid, 4 c.c. of standard phosphate were added to one tube and 5 c.c. to the other. After the addition of 2 c.c. of Na_2SO_3, 1 c.c. of hydroquinone, and 2 c.c. of ammonium molybdate, the tubes were made to the 15 c.c. mark, stoppered, mixed and allowed to stand. The readings were as follows:

AFTER 50 MIN.		AFTER 5 HRS.		AFTER 24 HRS.	
5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.
23.8	30	23.8	30	24.3	30

2. The above experiment was repeated with the addition of 1.5 c.c. of acid ammonium sulphate to each tube. The following readings were taken:

AFTER 75 MIN.		AFTER 3 HRS.		AFTER 23 HRS.	
5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.	5 c.c. std.	4 c.c. std.
22.7	30	24.1	30	25.2	30

3. Eight c.c. of acid ammonium sulphate were measured into each of two tubes. To one were added 3 c.c. and to the other 4 c.c. of standard phosphate followed by 2 c.c. of Na_2SO_3, 1 c.c. of hydroquinone, and 2 c.c. of ammonium molybdate and the volume made to 17 c.c. The readings were as follows:

AFTER 1.5 HRS.		AFTER 18 HRS.		AFTER 46 HRS.	
4 c.c. std.	3 c.c. std.	4 c.c. std.	3 c.c. std	4 c.c. std.	3 c.c. std.
Color too faint to read		22.1	30	22 4	30

RESULTS AND DISCUSSION

In Tables I and II are reported the results obtained by the described technic upon bloods from patients at the Chicago Municipal Tuberculosis Sanitarium.

TABLE I

PHOSPHORIC ACID COMPOUNDS IN WHOLE BLOOD IN TUBERCULOSIS
MG. OF H_3PO_4 PER 100 C.C.

INITIAL AND CASE NO.	SEX	CORPUSCLE VOLUME	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS $(NH_4)_2 SO_4$ FILTRATE	LECITHIN PLUS ACID SOLUBLE PHOSPHORUS	T-(L PLUS ASP)	INORGANIC PHOSPHORUS	N. T. A. CLASSIFICATION
H. B. 17022	♂	47.2	137.3	39.8	97.9	137.7	−0.4	14.7	Inc. B
P. G. 17058	♂	36.2	132.7	36.1	95.0	131.1	+1.6	14.4	F. A. B.
R. P. 17012	♂	43.4	130.2	40.9	86.7	127.6	+2.6	14.5	Mitral Stenosis Under Observation for TB.

TABLE II

PHOSPHORIC ACID COMPOUNDS IN WHOLE BLOOD IN TUBERCULOSIS
MG. OF H_3PO_4 PER 100 C.C.

INITIAL AND CASE NO.	SEX	CORPUSCLE VOLUME	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS TRICHLORACETIC ACID FILTRATE	LECITHIN PLUS ACID SOLUBLE PHOSPHORUS	T-(L PLUS ASP)	INORGANIC PHOSPHATE TRICHLORACETIC ACID FILTRATE	N.T.A. CLASSIFICATION
E. L. 17037	♀	41.4	115.2	31.0	81.1	112.1	+3.1	11.0	M.A.A.
A. P. 17032	♀	39.4	114.0	29.0	82.0	111.0	+3.0	10.4	M.A.A.
A. L. 17031	♀	39.9	118.5	36.9	82.1	119.0	−0.5	11.4	M.A.B.

TABLE III

INORGANIC PHOSPHORIC ACID MG. PER 100 C.C. WHOLE BLOOD

CASE NO. AND INITIAL	PER CENT OF CELLS	TRICHLORACETIC ACID FILTRATE				AMMONIUM SULPHATE FILTRATE		
		After 45 minutes	After 4 hrs.	After 18 hrs.	After 24 hrs.	After 4 hrs.	After 17 hrs.	After 24 hrs.
H. B. 17012	47.2	12.4	14.6	21.0	21.0	Color too faint to read	14.7	14.7
P. G. 17022	36.2	13.7	15.6	21.8	21.8	Color too faint to read	14.4	14.9
R. P. 17058	43.4	12.2	14.5	20.3	20.0	Color too faint to read	14.5	14.3

There is one disadvantage in determining the inorganic phosphate in an ammonium sulphate filtrate by the above technic, on account of the length of time necessary for color development. It has been pointed out by Bloor, Bell and Doisy that the inorganic fraction increases on standing.

Such an increase was observed here, but it was found to be greater in

TABLE IV

Distribution of Phosphoric Acid Compounds in Whole Blood and Plasma

Mg. of H_3PO_4 per 100 c.c.

Initials and Case No.	Sex	Corp. Volume	Total Phosphorus		Lecithin Phosphorus		Acid Soluble Phosphorus		Sum of L plus ASP		T-(L plus ASP)		Inorganic Phosphorus		N.T.A. Classification
			Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	
M. M. 18095	♀	40.4	112	34.4	33.5	22.3	78.3	11.1	111.8	33.4	+0.2	+1.0	13.0	10.8	M. A. B.
G. C. 18093	♀	40.2	110.4	31.6	30.9	21.1	76.7	10.9	107.6	32.0	+2.8	-0.4	11.7	10.4	M. A. B.
A. O. 17092	♂	45.6	120.9	26.9	34.7	17.0	84.5	10.3	119.2	27.3	+1.7	-0.4	10.9	8.8	M. A. B.

the trichloracetic acid filtrate than in the acid ammonium sulphate filtrate. This is indicated by the following experiments:

In one experiment, the trichloracetic acid and the ammonium sulphate precipitations were carried out immediately after the bloods were drawn. The tubes were prepared as usual and the readings made at various intervals. In Table III are tabulated the results.

In another experiment the inorganic phosphate determination was made as usual upon the acid ammonium sulphate filtrate immediately after preparation, and the amount found was 12.4 mg. The filtrate was allowed to stand at room temperature for 17 days and a second determination gave 15.6 mg.

The greater hydrogen-ion concentration of the trichloracetic acid filtrate may account for the difference in the increase of the inorganic phosphorus fraction.

In Table IV are recorded the results of phosphorus determinations made both upon the whole blood and the plasma of three patients. Trichloracetic acid was used as the protein precipitant.

REAGENTS AND SOLUTIONS

1. Ether-Alcohol Mixture—1 part of ether and 3 parts of 95 per cent alcohol, both redistilled.

2. Acid-Ammonium Sulphate—Saturated ammonium sulphate containing 15 c.c. of glacial acetic acid per liter. The C.P. ammonium sulphate was recrystallized twice from water and found to be free from all but traces of phosphates.

3. Trichloracetic Acid—This is a 20 per cent solution of trichloracetic acid.

4. Digestion Mixture—Equal parts of pure concentrated sulphuric and nitric acids.

5. Sugar Solution—1 per cent sucrose solution. To be made up fresh once a week.

6. Sodium Hydroxide—Approximately a 40 per cent sodium hydroxide solution prepared from sodium according to Bloor.

7. Phenolphthalein—0.5 per cent of pure phenolphthalein.

8. Dilute Sulphuric Acid—1 part of pure concentrated sulphuric acid and 3 parts of water.

9. Approximately an N/1 sulphuric acid.

10. Standard Phosphate—Stock standard contains 0.8340 grams of pure KH_2PO_4 per liter. The standard for use is made by diluting 25 c.c. of the stock solution to 500 c.c. One c.c. of the dilute standard is equivalent to 0.03 mg. of H_3PO_4.

11. Sodium Sulphite—20 per cent solution of sodium sulphite. This solution was made fresh at least twice a week.

12. Hydroquinone Solution—10.0 grams of hydroquinone per liter made slightly acid by the addition of 0.2 c.c. of pure concentrated sulphuric acid.

13. Ammonium Molybdate Solution—To 50 grams of pure ammonium molybdate dissolved in 300 c.c. of water are added 200 c.c. of water containing 75 c.c. of concentrated H_2SO_4.

All the water used in this work was collected and stored in glass water bottles.

When not in use the molybdate, phosphate, and hydroquinone solutions were stored in a refrigerator. The dilute phosphate solution was made fresh at least once a month.

SUMMARY

1. During the reduction of phosphomolybdic acid with hydroquinone an atmosphere of sulphur dioxide must be mantained to prevent oxidation by the air.

2. The sum of the lipoid and acid soluble phosphoric acid whether determined in the filtrate from the trichloracetic acid or ammonium sulphate precipitations, is practically equal to the total. The slight difference is attributed to experimental error. It might represent a fraction as nucleoportion which would be precipitated by either the trichloracetic acid or acid ammonium sulphate, and would also be insoluble in the ether-alcohol extract.

3. A procedure is described whereby a combination of the Bloor method with the Briggs modification of the Bell and Doisy method has been used for the estimation of total, lipoid and acid soluble, phosphoric acid in blood.

ADDENDUM

About the time this paper was sent to press one along similar lines appeared by Briggs.[3] In view of the fact that the somewhat different technic described above had already been used for seven months on a study on blood phosphates in tuberculosis, it was thought advisable to offer the same for publication.

BIBLIOGRAPHY

[1] Briggs, A. R.:   Jour. Biol. Chem., 1922, liii, 13.
[2] Bloor, W. R.:   Jour. Biol. Chem., 1918, xxxvi, 33.
[3] Briggs: Jour. Biol. Chem., 1924, lix, 255

A SIMPLE DEVICE FOR RELOCATING MICROCOLONIES*

By F. T. Breidigam and T. M. Chang, Battle Creek, Mich.

IN working with microcolonies such as those of B. acidophilus in a mixed plate, one often experiences the difficulty of relocating the particular colony or colonies under study when such a relocation is desired. Marking with a wax pencil has been our usual practice, but this method gives only the approximate whereabouts of the colony, and not the exact location. To find the colony again in the marked circle usually takes some time.

With a simple device such as that illustrated in the accompanying figure, any microcolony in a plate can be given its appropriate "street number," and be relocated whenever desired. It consists of a semicircular celluloid

disk, cemented or attached by means of a set of screws to a semicircular metallic frame. The disk is made of an ordinary process photographic film. One quadrant of the disk is ruled into small squares, each square equaling one sq. mm. In each square there is one figure in the left lower corner, and another one in the right upper corner, and by a systematic arrangement of figures, such as that illustrated in the sketch, each square will bear a different combination of two figures, and by this combination any micro-colony can be quickly relocated. The other quadrant is ruled so that it can be used as an ordinary bacterial counting plate.

When a colony is found which is to be relocated for further study, slip the disk over that half of the plate where the colony lies and in such a position so that the checkered quadrant will lie over the colony in question.
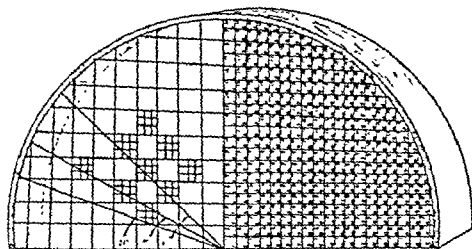
Fig. 1.

Be sure that the metallic frame is in close contact with the edge of the plate. With a wax pencil make a line on the plate along the diameter of the disk. Read the combination of figures in the square in which the colony is located, and this combination is the "address" of the colony. In reading the combinations of figures, it is best to read the figure in the lower left corner first, followed by the figure in the upper right corner. To relocate the colony put the disk over plate so that the marked line may lie along the diameter of the disk. Move plate around with the disk on top until the combination is found. Focus, and the colony will be seen.

This celluloid disk works excellently as a relocater of microcolonies, and serves also well as a convenient bacterial counting plate.

THE EFFECT OF HEAT ON STAINING PROPERTIES OF THE TUBERCLE BACILLUS[*]

By Thomas G. Hull, Ph.D., Kirby Henkes and Luella Fry, Springfield, Ill.

IT has become a common practice in many laboratories to subject to steam sterilizations specimens of sputum intended for examination for tubercle bacilli. The advantages of such a procedure are twofold, in that the specimen is not only easier to handle after the albuminous material is coagulated, but it is absolutely safe. Sputa without disinfectant (or with phenol 5.0 per

cent) may be coagulated by steam heat and preparations made from the coagulum on microslides. If a little alkali is added to the sputum, as twentieth normal sodium hydroxide or an alkaline disinfectant, as cresolis compositus, before the specimen is subjected to the autoclave, the albuminous material is entirely liquefied making it suitable for concentration tests.

Staining in these laboratories is performed after a modified method of Spengler.[1] The autoclaved sputums are smeared on microslides with a little Mayers egg albumen as a fixative, dried and heated gently. Carbolfuchsin is applied to steaming eight to ten minutes, the specimens decolorized in acid alcohol (3 per cent hydrochloric acid in 95 per cent alcohol) until faintly pink; then counterstained thirty seconds in a saturated alcoholic solution of picric acid. The red tubercle bacilli stand out very distinctly against the yellow background.

Several experiments were conducted to determine whether there was any difference in the results of examinations on raw and cooked specimens, and if not, how much heat could be applied to sputum before the tubercle bacilli lost their acid-fast properties.

1. *To Determine the Effect of Coagulation.*—Thirty-seven specimens of sputum were selected at random as they were submitted by various physicians. All specimens were in 5.0 per cent phenol. They were divided into two parts, the cheesy particles of one being smeared raw on microslides, while the other was heated at fifteen pounds pressure of steam for forty-five minutes, the smears then being made and stained as described above.

In every instance the two examinations checked, 9 specimens showing tubercle bacilli present and 28 being negative. Since these results are in accord with those of Jones[2] and other workers, no further comparisons were made.

2. *To Determine How Long Sputum May Be Cooked.*—A pooled specimen of sputum was heated in the autoclave on successive days whenever opportunity permitted, smears being made and examined after each heating. On the twelfth heating, with a total of 8.5 hours, the organisms began to hold the stain weakly; at the fourteenth heating, with a total of ten hours at an average of eight pounds steam pressure, the tubercle bacilli were no longer acid-fast.

3. *To Determine the Effect of Heat in the Presence of Acid and Alkali.*—A pooled specimen of positive sputa was divided into seven parts: Three received equal amounts of sterile water, two received equal amounts of twentieth normal NaOH and two equal amounts of twentieth normal HCL. One of each of the above groups was left at room temperature, one of each heated at successive intervals in the autoclave and the last one with the sterile water placed in the incubator at body temperature. After a period of eighteen days all of the specimens at room temperature and one specimen in the incubator showed acid-fast organisms present. Fourteen heatings over the same period were given the other specimens under conditions similar to those in Experiment 1. After the eighth period in the autoclave the specimen containing twentieth normal HCL no longer showed acid-fast organisms. The

other two specimens—neutral and alkaline—showed acid-fast organisms to the end of the experiment.

4. *To Determine the Effect of Dry Heat.*—From a pooled specimen of positive sputa (previously cooked 45 minutes in autoclave) smears on ten glass slides were made. These were air-dried and placed in the hot air oven at a temperature of 154° C. to 156° C. Every ten minutes a slide was removed for staining purposes. At the end of the period—one hour and forty minutes—the organisms which had been subjected to the dry temperature for the entire time showed no indication of losing their acid-fast property. This indicates that haste in drying a smear of sputum for staining purposes, with incidental heating, does not injure it for the acid-fast stain.

5. *To Check the Accuracy of Tests Made on Specimens Heated with an Alkali.*—It has been shown that an alkaline disinfectant such as cresolis compositus[3] is considerably more effective in disinfecting sputum than is phenol. For this reason it has been customary in these laboratories to use the former material. When sputum is subjected to heat in such a solution it liquefies almost completely. After two or three hours standing, smears made from the sediment show tubercle bacilli in considerable numbers. There is the possibility, however, that when the organisms are few in number in a given sputum they will not sediment sufficiently by natural gravitation to be found in a smear of material from the bottom of the vial.

To determine the accuracy of the above method, the results of examinations made as described were checked with concentration tests. Sputa in cresolis compositus were sterilized in the autoclave and allowed to stand for two or three hours. The supernatant fluid was poured off and saved while smears were made from the sediment. Since the specific gravity of the tubercle bacillus is 1010 to 1080[4] there is the possibility that a solution too strongly alkaline will not allow the organisms to fall to the bottom. To reduce the specific gravity of the mixture, an equal part of alcohol was added before centrifuging (Loeffler used an equal part of alcohol containing a little chloroform). After twenty minutes centrifuging at high speed, the supernatant liquid was poured off and smears made of the sediment in the routine manner.

Of 490 duplicate examinations, 474 checked as being either positive or negative in each (102 positive and 372 negative), while 16 were at variance. Nine tests were positive by the direct smear or gravity method and negative in the alcohol centrifuge method, while seven were negative by the first method and positive by second. It would seem, therefore, that when sputum is dissolved in cresolis compositus 3 per cent solution, tubercle bacilli will sediment in the course of two or three hours by natural gravity, making the results of the tests as accurate as when the centrifuge is used.

CONCLUSIONS

1. Specimens of sputum subjected to steam sterilization are much easier and safer to handle and give as reliable results as do raw sputa.

2. Sputum may be sterilized as long as ten hours at an average of eight pounds steam pressure before the organisms lose their acid-fast properties.

3. In the presence of twentieth normal HCL six hours of sterilization sufficed to render the organisms no longer acid-fast. Twentieth normal NaOH apparently did not affect the acid-fast properties of the organisms either at room temperature or in the autoclave after repeated heatings.

4. Dry heat for one hour and forty minutes at 154° C. to 156° C. did not affect the acid-fast properties of the organisms.

5. Concentration tests may be made by liquefying sputum in the autoclave with cresolis compositus and allowing the tubercle bacilli to sediment by gravity.

Credit is due Mr. Alfred S. Harkness for his assistance in this work.

REFERENCES

[1]Spengler: Deutsch. med. Wchnschr., 1907, xxxii, 337.
[2]Jones: Jour. Lab. and Clin. Med., 1921, vi, 41.
[3]Park and Williams: Pathogenic Micro-organisms, edition 6, p. 392.
[4]Nebel: Arch. f. Hyg., 1903, xlvii, 57.

A SIMPLE AND RAPID METHOD FOR THE PREPARATION OF POLYCHROME METHYLENE BLUE AND THIAZIN RED. A RAPID METHOD FOR STAINING FROZEN SECTIONS WITH THIAZIN RED*

By Frederick Proescher, M.D., and Albert Paul Krueger, A.B.,
San Jose, California

THE polychrome methylene blue solution introduced by Unna for the staining of mast cell granules has found a wide range of usefulness in histologic technic. Originally empirically prepared by Unna[1] without knowledge of its chemical composition, many attempts were made by biologists and chemists to isolate and identify the specific component which gave to the solution its characteristic staining properties.

Romanowsky's[2] observation on the staining of the malarial parasite's chromatin substance gave the first impetus for inquiry into the composition of various commercial methylene blues as well as into that of polychrome methylene blue. This investigator found that eosin-methylene blue would sometimes stain certain cellular constituents of the Plasmodium malariae (later recognized as the chromatin substance) a distinct red color. He further determined that with a pure methylene blue the granules could not be stained. The particular brand of commercial methylene blue used together with the age of the solution were evidently the factors involved in production of the specific staining effect. The inconstancy of results obtained remained unsolved by Romanowsky and his method was almost forgotten.

Later on Ziemann,[3] in a painstaking investigation, tried to overcome the

difficulties of the original Romanowsky method and found that by using methylene blue medicinale (Höchst) with eosin in certain definite proportions, a constant and distinct chromatin stain could be effected. He proffered the opinion that the specific dye responsible for the chromatin staining, and formed by mixing eosin and methylene blue together, was the so-called eosinic-acid-methylene blue.

At the same time, Nocht[4] started his investigation on the Romanowsky method and came to the conclusion that not the eosinic-acid-methylene blue but certain contaminations of commercial methylene blue in combination with eosin were essential for staining of the chromatin granules. He concluded also that Unna's polychrome methylene blue contained these decomposition products of methylene blue in large quantities. By using a neutralized solution of Unna's polychrome methylene blue (to eliminate effects due to the high alkali content) and mixing this with eosin, he obtained constant and distinct staining of the chromatin.

Ziemann[5] simultaneously reported that the addition of sodium borate (2-4 per cent) to methylene blue (Höchst) plus eosin gave constant results. Thus both methods were independent of the commercial brand of methylene blue and eosin used.

The specific dye concerned still remained unknown. Nocht's[6] further investigations demonstrated that a red derivative was formed by prolonged contact of methylene blue with alkali at room temperature, the action proceeding more rapidly at moderate heat. This substance, responsible for the specific staining of the protozoan nucleus, he designated for the time being as "Rot aus Methyleneblau." He gave as a mode of detecting its presence the following test: Extraction with $CHCl_3$ in which the dye is soluble with the production of a fiery red color throughout the extracting medium. Reuter[7] disputed that Nocht's "Rot aus Methyleneblau" was the specific factor in chromatin staining and contended that eosinic-acid-methylene blue alone was capable of producing the same staining effect. But his contention was erroneous and was disproved by Michaelis and Ziemann.

Finally Michaelis[8] pointed out that Nocht's "Rot aus Methyleneblau" was likely the so-called Methylenazur first prepared by the chemist Bernthsen.[9] Bernthsen had advanced the opinion that Methylenazur was simply an oxygen addition product of methylene blue (a methylene blue sulphone), and had attributed to it the following formula:

$$
\begin{array}{ccc}
 & C_6 \quad H_3 \text{------} N(CH_3)_2 & \\
HN & & SO_2 \\
 & C_6 \quad H_3 \text{------} N(CH_3)_2 &
\end{array}
$$
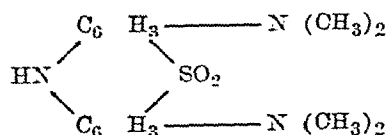
However, Bernthsen's formula was disproved by Kehrmann[10] who presented conclusive evidence that the oxidation did not lead to an addition of oxygen at the bridge of the methylene blue, but rather was due to a demethylation of one or two methyl radicals of the side chains.

CHEMISTRY OF THE METHYLENE BLUE AND ITS OXIDATION PRODUCTS

The following discussion is perhaps in order as furnishing a basis for the proper understanding of synthesis and oxidation products.

(a) *Synthesis of Methylene Blue.*—Starting with ammonia, two hydrogen atoms are replaced by phenyl groups:

$$NH_3 \longrightarrow HN \Big\langle { C_6 H_5 \atop C_6 H_5} \qquad \textit{Diphenylamine}$$
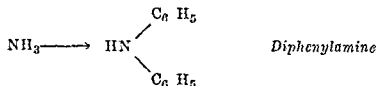
This chain becomes a closed ring by introduction of sulphur:

$$HN \Big\langle {C_6\ H_4 \atop C_6\ H_4} \Big\rangle S \qquad \textit{Thiodiphenylamine}$$

Thiodiphenylamine is known as Thiazin and forms the nucleus (chromophore) of all the methylene blue series of dyes.

The addition of two amino groups (auxochromes) in the side chains produces the simplest dye:

$$HN \Big\langle {C_6\ H_3 \text{———} NH_2 \atop C_6\ H_3 \text{———} NH_2} \Big\rangle S \qquad \textit{Leuco Base of Thionin}$$
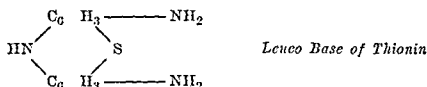
Substitution of the hydrogen atoms of the amino groups in the side chains by methyl radicals results in the formation of the so-called leuco methylene blue (or methylene white):

$$HN \Big\langle {C_6\ H_3 \text{———} N\ (CH_3)_2 \atop C_6\ H_3 \text{———} N\ (CH_3)_2} \Big\rangle S \qquad \textit{Leuco Methylene Blue}$$
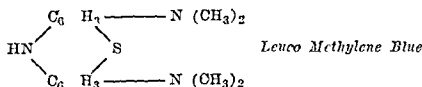
Treatment of this compound with an acid, e.g. HCl, gives methylene blue:

$$N \Big\langle {C_6\ H_4 \text{———} N\ (CH_3)_2 \atop C_6\ H_3 \text{———} N\ (CH_3)_2} \Big\rangle S \qquad \textit{Methylene Blue}$$
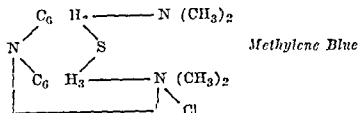
(b) *Oxidation Products of Methylene Blue.*—Oxidation of methylene blue results in the partial demethylation of the side chains, these latter being replaced by hydrogen atoms. The chief products of this demethylation process are trimethylthionin and asymmetrical dimethylthionin. The ratio of the dyes formed depends on the mode of preparation, the nature of the oxidizing agent being decidedly influential. The methyl groups split off are very likely oxidized to methyl alcohol and formaldehyde. It is possible that the demethylation does not stop with formation of dimethylthionin, but may proceed to monomethylthionin or thionin. These substances have

not yet been identified among the reaction products. The isolation of dimethylthionin by Bernthsen points to a complete destruction of the methylene blue molecule.
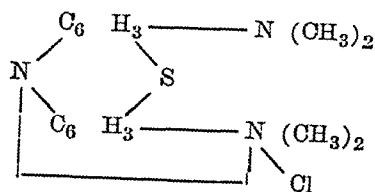
Chlorhydrate of Methylene Blue
(Blue)
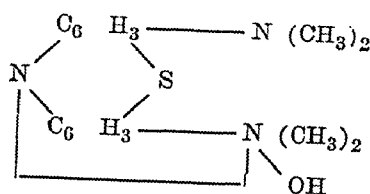
Free Base of Methylene Blue
(Blue)

Demethylation derivatives
(Methylenazures)
Trimethylthionin)
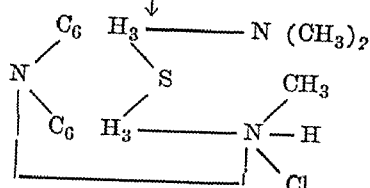
Chlorhydrate of Trimethylthionin
(Blue)
Water-Soluble
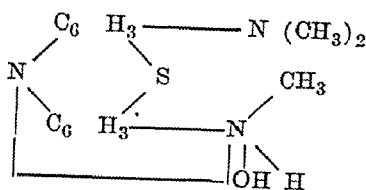
Free Base Hydrate of Trimethylthionin
(Blue)
Water-Soluble
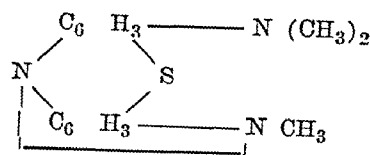
Free Base Anhydride of Trimethylthionin
(Red)
$CHCl_3$-Soluble

Dimethlythionin
(Asymmetrical)
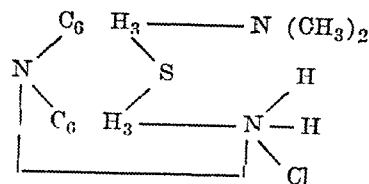
Chlorhydrate
(Blue)
Water-Soluble
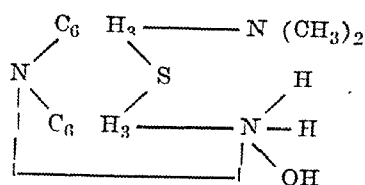
Free Base. Hydrate
(Blue)
Water-Soluble
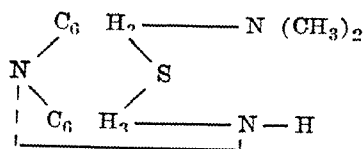
Free Base. Hydrate
(Red)
$(C_2H_5)_2O$-Soluble

The ammoniacal character of methylene blue is changed by the oxidation process, and the resulting trimethylthionin and dimethylthionin derivatives have the properties of amines. While the free base of methylene blue is not precipitated by alkalies, and no color change occurs, the bases of tri- and dimethylthionin are precipitated as red colored anhydrides. These are insoluble in concentrated alkalies, but dissolve in chloroform or ether, imparting to the solution a cherry red color. Extraction of the ether or chloroform solution with water produces the blue hydrates which are soluble in the water.

Unna[11] proposed to include under the name, "Thiazin Red," the free bases of trimethylthionin and dimethylthionin. It may be more correct, however, to term these dyes "Trimethyl and dimethyl thiazin red." Commercial methylenazur is a mixture of trimethyl and asymmetric dimethylthionin, the latter predominating. Polychrome methylene blue solution contains besides both these substances unchanged methylene blue, some leucomethylene blue, and the decomposition products of methylene blue.

Polychrome Methylene Blue solution, e.g., thiazin red, has the peculiar property of staining certain cellular elements metachromatically; metachromatic referring to the staining of various cellular structures with different colors by a single chemical substance. An aqueous solution of polychrome methylene blue stains amyloid substance, mast cell granules, mucus and chondrium red, instead of blue. The above mentioned cellular elements must take up the anhydrides of the thiazin reds. The same phenomenon is observed if a pure watery solution of trimethylthionin and dimethylthionin is used.

It is a question whether the metachromatic properties of the thiazin bases can be explained as caused by differences in the color of their hydrates and anhydrides, or if there exists a tautomerie, e.g., intramolecular rearrangement of the hydrates and anhydrides due to different solvents.

PREPARATION OF POLYCHROME METHYLENE BLUE SOLUTION AND THIAZIN RED FROM METHYLENE BLUE BY OXIDATION WITH SODIUM PEROXIDE

For the preparation of polychrome methylene blue as well as thiazin red we found that Na_2O_2 could be used to considerable advantage in place of strong and weak alkalies, $K_2Cr_2O_7/H_2SO_4$, etc. The oxidation is very rapid and is in every way superior to that produced by the above mentioned agents. It may be carried on in an aqueous solution or may be performed in ethyl alcohol, acetone or chloroform.

Any good commercial brand of zinc chloride-free methylene blue (preferably "medicinale") may be used. In the event that a very pure polychrome methylene blue solution is desired, the methylene blue is best subjected to several preliminary recrystallizations from alcohol.

As stated above, the polychrome methylene blue solution, no matter what oxidizing agent is used in its preparation, contains varying amounts of methylene blue, leucomethylene blue, tri- and dimethylthionin (thiazin reds) and other decomposition products of methylene blue, in an alkaline solution of varying strength. The thiazin reds are most important for staining effects.

By oxidizing 100 c.c. of a 1 per cent methylene blue solution with different amounts of Na_2O_2 (5-100 mg.) the percentage of thiazin reds can be accurately controlled. Increasing amounts of Na_2O_2 cause the solution to change from a weak bluish red to a deep cherry red color.

A polychrome methylene blue solution suitable for pathologic work is obtained by using 20-30 mg. Na_2O_2 to 100 c.c. 1 per cent methylene blue solution. The Na_2O_2 is quickly weighed out to preclude any extensive reaction with the water vapor of the atmosphere and is added to the methylene blue solution. The mixture is immediately placed in a water-bath at 100° C. for fifteen minutes or may be boiled over a Bunsen flame for five minutes.

If a neutral solution of polychrome methylene blue or one of a particular alkalinity is desired, to meet the needs of special staining methods, the free alkali formed during the oxidation process may be neutralized with exact amounts of N/10 HCl. The necessary amount of acid is calculated from the following: 5 mg. Na_2O_2 : 1.18 c.c. $\frac{N}{10}$ HCl. HCl is for this procedure decidedly superior to organic acids, e.g., HCOOH, CH_3COOH, etc.

Where a stable polychrome methylene blue is to be made up, the alkali should be accurately neutralized since in alkaline solution, under the influence of atmospheric oxygen, unoxidized methylene blue is converted to thiazin reds which are insoluble in the presence of NaOH and precipitate, rendering the stain unfit for use. It is well to keep the neutral polychrome methylene blue solution in a paraffined bottle, otherwise alkali may dissolve out of the glass and produce oxidation.

PREPARATION OF THE THIAZIN REDS (METHYLENAZUR)

Since the specific staining properties of polychrome methylene blue are dependent upon the thiazin reds, it is obviously preferable to employ a solution containing these dyes only in place of a mixture of these substances in varying amounts with undesirable decomposition products of methylene blue.

Following is a simple and rapid method for the preparation of thiazin reds:

Fifty grams of methylene blue are dissolved in 200 c.c. water (using a 500 c.c. beaker) and the solution heated to 75-80° C. Five grams of Na_2O_2 are dissolved in 20-30 c.c. water and quickly added to the warmed methylene blue. The reaction takes place immediately with resultant precipitation of the free thiazin bases as large bluish-red masses exhibiting a peculiar metallic luster. The solution when cooled is filtered through a Buchner funnel and the precipitate dried at 37° C. After 24 hours at this temperature it is advisable to break up the large lumps in a mortar and to dry again. The fine powder obtained is completely dried over $CaCl_2$ or concentrated H_2SO_4 and kept in a tightly stoppered bottle.

A suitable staining solution is prepared by dissolving 0.5 gm. of the dried bases in 100 c.c. distilled water. The weighed quantity of dye is ground in a mortar with small amounts of water until completely taken up. The solution is then diluted to 100 c.c. and filtered. A deep azure-blue solution is obtained which keeps perfectly in a paraffined bottle.

STAINING OF FROZEN SECTIONS WITH THIAZIN RED

The above described aqueous solution of thiazin red is well adapted to the staining of frozen sections. However, when used for this purpose, it is best to employ 1 per cent acetic acid instead of distilled water as a solvent. Five-tenths gram thiazin red is dissolved in 100 c.c. 1 per cent acetic acid (made by diluting 1 c.c. glacial acetic acid to 100 c.c.).

This slightly acid solution gives very sharp differentiation of the nuclei of epithelial cells and leucocytes. Connective tissue remains either colorless or is stained very slightly blue or metachromatic violet. Sections remain ten to twenty seconds in the staining solution, are washed with water and mounted in glycerine or better levulose syrup. Immersion of the section for even five to ten minutes in the staining solution will not result in overstaining, for a thorough washing in water gives an excellently differentiated picture. Sections may be kept some time if mounted in thick levulose syrup and framed with asphalt lack or paraffin

The procedure described here is especially suited to formalin fixed material. Frozen sections cut from tissue which has not been previously treated with formalin, should be immersed in 5 per cent formalin solution for ten to twenty seconds and placed directly in the staining mixture without washing in water.

SUMMARY

1. The historical development of polychrome methylene blue together with the general chemistry of methylene blue and its oxidation products is outlined.

2. A rapid and convenient method for the preparation of polychrome methylene blue solution and thiazin reds from methylene blue by oxidation with sodium peroxide is given.

3. A discussion of metachromasie and the application of a solution of thiazin reds to frozen section technic conclude the paper.

BIBLIOGRAPHY

[1]Unna, P. G.: Monatschr. f. Proktische Dermatol., 1885, xii.
[2]St. Petersburger med. Wchnschr., 1891, Zur Frage der Parasitologie und Therapie der Malaria. St. Petersburg, 1891.
[3]Ziemann: Ueber Malaria und der andere Blutparasiten, Jena, Fischer, 1898.
[4]Nocht: Centralbl. f. Bakt., 1898, xxiv.
[5]Ziemann: Centralbl. f. Bakt., 1898, xxiv. No. 25.
[6]Nocht: Centralbl. f. Bakt, 1899, xxv, Nos. 21-22.
[7]Reuter: Centralbl. f. Bakt., 1901, xxx, No. 6.
[8]Michaelis, L.: Centralbl. f. Bakt., 1901, xxix, No. 19.
[9]Bernthsen: Ann. d. Chem., 1885, vol. 229-230, p. 169.
[10]Kehrmann, F.: Berichte der deutschen chemischen Gesellschaft, 1906, ii, 1403.
[11]Unna, P. G.: Centralbl. f. Bakt., Parasitenkunde, 1922, Heft 2.

A TETANUS TOXIN FILTERING APPARATUS[*]

By Edward Redowitz, Glenolden, Pa.

IN the routine production of tetanus toxin, the process of filtration is one of the most painstaking and needlessly difficult tasks. The handling of living tetanus cultures with the toxin, and the forcement of the sticky fluid through filter candles present the most objectionable features of the operation. Some laboratories seek to obviate the latter difficulty by several preliminary filtrations. This requires more manipulation of the culture than would be desirable and at the same time may reduce the potency of the toxin. The apparatus described in this paper was designed with all these points in mind, and for the past three years has been used with marked success.

The machine is simple in construction and easy to operate. The culture must be poured only once, and within two or three hours, using a triple machine, thirty or forty liters of toxin can be filtered without the danger and delay of other methods. The main feature of the apparatus is the use of percolators or large earthenware funnels as sheaths for the candles. The candles are encased in the lower, narrow part. Above the candles in the upper portion, filters consisting of layers of paper pulp serve to remove most of the organisms and slimy substance before the filter candles are reached.

The apparatus may be set up on any ordinary wooden laboratory table, as illustrated. Holes are made in the table to fit the lower ends of the percolators. A wooden stand, six inches high, with larger holes, is attached to the table. This will hold the percolators firmly in position. All the parts are durable. The preparation of the paper pulp requires a little time, but it can be made in large quantities and kept ready for use in a suitable container with a tight lid.

The number of percolators to be used will be governed by the quantity of toxin to be filtered. One percolator set up complete will hold about four liters of fluid, and, by means of water vacuum, enough toxin can be drawn through to fill two nine liter bottles. The pulp and the filter candle will then become clogged and should no longer be used.

After using the apparatus the percolators with their contents can be placed in a can and autoclaved. The rubber stoppers holding the candles should be removed from the percolators, and to save them from melting, must be autoclaved in a vessel containing water. No other connections need autoclaving.

The parts necessary for one percolator are:

1. A cone shaped stoneware percolator.
2. A Berkfeldt or a Mandler filter candle 2 × 10 inches.

3. A one-hole rubber stopper size No. 10 to hold filter candle in percolator.

4. Galvanized wire screen with a rim, for bottom of percolator.

5. Filter paper cut round to fit on wire screen.[4]

6. Paper pulp soaked in 0.5 per cent solution of phenol.

7. Filter paper cut round to fit on top of paper pulp.

8. Round galvanized wire screen to be used as weight on top of last layer of filter paper.

9. Two pieces of rubber pressure tubing connected to glass tube elbows that fit in a rubber stopper.

10. A sterile bottle.

<center>PREPARATION OF PULP</center>

Ordinary filter paper is torn in long strips, two or three inches wide, placed in an agateware can. It is then covered with 0.5 per cent solution of phenol, and allowed to stand overnight. The next morning the phenol solu-
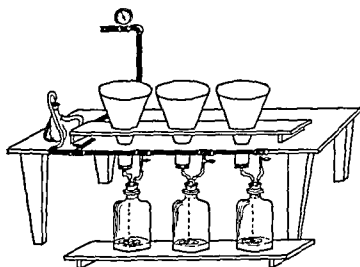
Fig. 1.
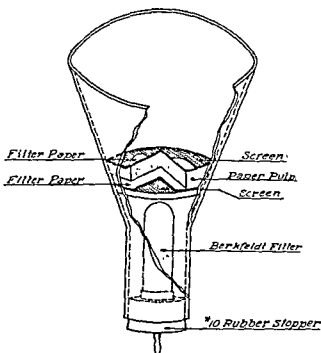
Fig. 2.

tion is poured off completely. The wet paper is then ground in a mechanical meat grinder, or if a meat grinder is not available, the paper can be rubbed into pulp on an improvised grater made, for example, of a large galvanized can lid with holes punched so that the sharp edges stick up. The wet pulp is then partially dried in an incubator.

The proper degree of dryness is important, for if the pulp is too wet, it will pack down so tight in the percolator that it will interfere with the free flow of the fluid. On the other hand, if the pulp is too dry, it will not be an effective filter.

The pulp is spread out on paper in an incubator and allowed to remain for one or more days, until it becomes so nearly dry that an ordinary pressure of the hands will not squeeze fluid out of it, but it must feel moist to the hand. When pressed and moulded, it should hold together in a lump.

If it crumbles, it is too dry. The finished pulp is placed in an agateware can with a tight lid, and kept in a cool place.

SETTING UP THE APPARATUS

After the pulp has been prepared and all the necessary parts assembled, the apparatus may be set up when needed. The only parts necessary to be sterile: filter candle (2), rubber stopper (3), rubber tubing (9), and receiving bottle (10). The filter candle may be wrapped in paper and autoclaved, and all rubber stoppers and rubber tubing can be sterilized by boiling in a suitable vessel just before use.

The stem of the sterile filter candle (2) is passed through the sterile rubber stopper (3),which is inserted securely into the narrow end of the percolator. The percolator is then placed in position. The paper filter is now arranged above the filter candle, the parts being added in the following order: (4) The wire screen. (5) Two layers of filter paper. (6) Three or four inches of paper pulp, packed tightly by means of a small heavy glass bottle with a round bottom or some other flat bottomed instrument. (7) Another double layer of filter paper and finally the wire screen. (8) The filter candle is now connected by means of sterile rubber tubing with the vacuum tube and the sterile bottle. The percolator is filled by pouring the culture slowly along the side, the vacuum pump is started.

Note: With from twenty to twenty-eight inches of vacuum, nine to eighteen liters of toxin can be drawn through each filter.

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EDITORIALS

The Reaction to Syphilitic Infection

THE evolution of methods for the isolation and growth in pure culture of the Spirochete pallida inaugurated an intensive study of experimental syphilis in the rabbit, the results of which are now beginning to assume proportions of some significance.

Predominant workers, of whom there are many in this field, are Brown and Pearce, whose contributions to the subject have opened up avenues of investigation which have a direct and definite application to the clinical recognition and handling of this infection.

In spite of the antiquity of syphilis and the intensive and extensive study to which its manifestations have been subjected, there are still many phenomena associated with this disease the mechanism of which remains to be elucidated; for example, the factors influencing the pleomorphism of its clinical manifestations, or those determining the incidence of neurosyphilitic involve-

ment; what determines the latent periods of the infection—which are undoubtedly more frequent than has hitherto been supposed—and the like, answers to some of which problems are beginning to appear possible.

A factor of importance as related to the widespread incidence of syphilis and a possible explanation of those cases in which meticulous examination has failed to demonstrate the primary lesion, is found in the observation that highly virulent strains of Spirochete pallida may and can penetrate the normal, unbroken mucous membrane of the rabbit with resultant generalized syphilis but without the prior production of a gross lesion at the portal of entry.[1]

The clinical failure to prevent the disease in olden days by excision of the chancre and the failure of prophylactic measures when unduly delayed are explained by the experimental demonstration of the rapidity with which the spirochetes migrate and are disseminated from the site of invasion, as it has been shown that spirochetes are constantly present in the regional lymph nodes within forty-eight hours after inoculation and long before there is any evidence in the slightest degree of abnormality at the site of invasion.[2]

It has also been demonstrated that the pathway by which this dissemination takes place is primarily the lymphoid structure, though spirochetes have also been isolated from the blood in the rabbit, seven, ten, and thirty days after intratesticular inoculation, periods corresponding to twenty-six, twenty-three, and three days prior to the appearance of the initial lesion. Invasion of the blood and lymphoid system, therefore, occurs early in the incubation period of the disease.[3]

The relation of the lymphoid tissues to spirochetal infection is very interesting and of great practical importance. The adenopathy of syphilis has long been familiar but its importance and exact relation to the disease has, perhaps, not been as well appreciated and certainly not previously as clearly demonstrated. The importance of the lymph structures as avenues for the early dissemination of the spirochetes in syphilis has been shown by Eberson,[3] and by Brown and Pearce,[2] who emphasize the significance and importance of these structures as "reservoirs" of syphilitic infection. The constancy of this early localization of the spirochetes in the lymph nodes, their presence as early as five days after inoculation, and the fact that such localization serves as an effective focus for the maintenance and systemic dissemination of the disease, constitute important observations of definite practical application. Of even greater importance, however, is the demonstration that lymph node infection may survive and persist for long periods during which the disease is latent and entirely asymptomatic, which has been indisputably shown in the rabbit.[2]

That similar conditions obtain in the human being, has been shown by the studies of Engman and Eberson[4] concerned with the infectivity—as determined by rabbit inoculation—of the blood, semen, nasal washings, and lymph nodes of a series of cases latent from one to forty years, these being selected from a series of five hundred syphilitics, 15 per cent of whom were latent and asymptomatic.

In five of fourteen cases viable and virulent spirochetes were found on

the examination of a single lymph node, and in two out of seventeen cases spirochetes were found in the semen. How many more positive findings might have resulted from repeated examinations it is impossible to hazard. The demonstration of the spirochetes in the semen is an observation of decided interest.

Comparable findings are reported in the rabbit, in which, "though spontaneously clinically recovered," and showing no clinical or pathologic evidences of infection other than an enlargement of the popliteal nodes, viable and virulent spirochetes were found in these structures from which observation it was suggested that the possible seats of predilection for spirochetes during the latent periods and the chief sources or reservoirs of infection were the lymphoid structures, either massed as nodes or as simpler perivascular lymphatics.[5]

Experimental evidence is also available to indicate that the invasion of the central nervous system may take place through the lymphatic system[6] and, as is now a matter of clinical knowledge, that such invasion may take place early in the disease.

Recalling the fact that, while there is no unanimity in regard to satisfactory criteria for the cure of syphilis, a frequently adopted standard has been the success or failure of reinoculation, Chesney and Kemp[7] suggest the possibility of the utilization of the lymphoid tissues as test materials in determining the efficiency of therapeusis.

The problem attacked by these investigators was to determine if it were possible through the use of arsphenamine to render syphilitic rabbits free from spirochetes as determined by lymph node transfer, and whether such "cured" rabbits were susceptible to a second inoculation with the same strain.

Their conclusions follow:

"1. Six doses of arsphenamine (10 milligrams per kilo) 127 days after infection are sufficient to render the popliteal lymph nodes incapable of transmitting the infection to normal animals.

"2. Such rabbits, as judged by the absence of local lesions, are refractory to inoculation.

"3. Untreated rabbits were also refractory to inoculation.

"4. The refractory state is probably due, either to an acquired immunity, or to the persistence of living foci elsewhere in the body."

Reinfection or superinfection, in the light of the experimental evidence available, can no longer be looked upon as indisputable evidence of the prior total eradication of syphilis, for experimental demonstration of superinfection in the rabbit has been achieved and explained on the assumption that the resistance acquired as a result of infection with an organism of low virulence. may never reach the point of effectual protection against an organism of high virulence.[8]

Experimental superinfection is also reported by Eberson,[9] who concludes that a state of resistance does not necessarily prevent reinoculation but appears to favor a localization of the organism in the tissues without manifest lesions. Isolated clinical reports of probable reinfection can be found in the current literature, the most clear-cut case recently available being that re-

ported by Shinkle[10] in which a typical acquired chancre containing Spirochete pallida was demonstrated in a negro aged sixteen with a history of congenital syphilis corroborated by the presence of clinical and laboratory evidence of general paralysis.

The mechanism of reinoculation or superinfection is closely related to immunity in syphilis which has not been neglected in experimental studies. Whether or not a second (superinoculation) inoculation in the rabbit is capable of further participation in the disease or, because of acquired resistance through the first infection, limited to the production of local reactions, has been studied by Brown and Pearce.[11]

Their conclusion was that the ultimate lesions are probably due to the combined action of both strains.

According to Eberson,[12] the elaboration of syphilitic antibodies appears to be at a maximum, late in the disease, predisposing to a condition of latency without demonstrable lesions, though spirochetes could be found, the lesions following inoculation with such "latent" strains showing a definite tendency toward a chronic proliferating character.

A possible explanation of this tendency of syphilitic lesions toward involution *in situ*, as advanced by Eberson and Engman[13] lies in the probable formation of local antibodies which slowly destroy or tend to repress the activity of the spirochetes.

Chesney has studied the influence of age, sex, and method of inoculation on the course of syphilitic infection in the rabbit and concludes that intradermic inoculation is less prone to cause generalized syphilis in the rabbit than intratesticular inoculation; that the primary inoculation in females gives rise to a less marked reaction than in males; that young rabbits were more prone to marked initial lesions with slight tendency toward the postponement of general lesions which were less severe in character and extent than in older animals. He also found that the suppression by castration of the initial lesion did not lead to the increased incidence or early appearance of subsequent generalized lesions during an observation period of ninety days and that intradermal inoculation with little or no local reaction might give rise to general invasion as evidenced by positive lymph node transfer even though no local lesions might be observed.[14]

Studies of animal resistance to syphilis have opened up interesting by-paths among which may be noted the possible relationship and influence of the endocrine glands to the reaction to syphilis.

It is well known that the endocrine balance is subject to variations during pregnancy and studies in the human being have shown an association between the phenomena concomitant with pregnancy and the reaction to syphilitic infection which are borne out by the results of observations in experimental syphilis in the rabbit in which it has been found that the reaction to genital infection coincident with conception varies from the reaction in the normal animal similarly inoculated, and that this difference, moreover, extends well into the period of lactation.[15]

A similar study by Moore[16] in the human being may be thus summarized:

If impregnation and infection practically coincide, or if infection occurs during pregnancy, the patient may develop the usual manifestations of syphilis which, however, are much milder than if the infection is independent of pregnancy.

Some cases, when infection occurred about the time of impregnation, failed entirely to develop the usual early lesions of syphilis, and in a few cases (three out of two hundred), the response to infection acquired at the beginning of or during pregnancy was markedly altered, the usual time elapsing between the primary and secondary lesions being much prolonged, or the interval between the secondary and tertiary lesions being much shortened. The protection afforded by pregnancy in some cases was shown to be prolonged and the suggestion is advanced that the explanation might lie in some change in the endocrine system or in various changes in the balance of metabolism dependent upon or associated with the occurrence of pregnancy. These theories, however, as well as that of the possible influence of the chorionic ferments—the very existence of which has yet to be proved—remain to be demonstrated.

That some relation exists between endocrine efficiency and the phenomena of syphilis appears probable on experimental and inferential grounds, but just what this relation may be and what its modus operandi is, as yet, is unproved.

That the endocrine balance is, at times, disturbed in pregnancy is a matter of fact and there may be some relation between this imbalance and the variations in pregnancy of the reaction to syphilitic infection. Moreover, experimental data show that the status of the endocrine glands varies with different stages of syphilitic infection, and that they appear to be on a higher plane of functional activity (thyroids, parathyroids, adrenals, and thymus); still further, the course of the disease can be modified by operative interference with the glandular system, or by the administration of chemical agents effecting changes in the endocrine glands and lymphoid tissues, findings which may have some bearing on the abnormalities present in congenital syphilis.[17]

As neoplasms are characterized by lawless and irregular growth, and as certain of the phenomena associated with normal growth are related to endocrine structures, the occurrence of a spontaneous neoplasm in the scar of an old scrotal syphilitic lesion in the rabbit is of interest.[18]

The protean character of the lesions of syphilis has more than once given rise to the suggestion that there were possibly varying strains of Spirochete pallida with different predilections. While definite evidence for or against this supposition is yet to be adduced, and the question is still an open one, it is beyond cavil that variations in the clinical manifestations of syphilis may also be influenced by other factors, such as the reacting mechanism of the host and the disease-producing properties of the infecting strain.

Experimental study of syphilis in the rabbit has shown beyond question a relation between the defensive reaction and mechanism and the degree

of local reaction at the site of invasion, and studies of the effects consequent upon a modification by various means of this local reaction have shown that it is, in this manner, possible to vary the types of general reaction produced and to protect certain groups of structures—bones, for example—by allowing the testicular initial lesion to progress to a given point.[19]

It is held possible, therefore, that a modification of the defensive reaction influences the clinical variations of the disease while also admitting the possibility that biologic variations in the infecting organisms may contribute still further to variations in the type of syphilis produced.

The idea of an inhibitory influence has more than once been suggested as an explanation of the various phenomena of syphilitic infection and such effects have been strikingly shown in experimental infections in the rabbit in which scrotal or testicular lesions giving extremely marked reactions at the site of inoculation have been accompanied by total absence of the general manifestations of the disease.

An investigation conducted to determine the influence of a local reaction in one part of the body to manifestations of the disease elsewhere by means of a reduction or suppression of the local reaction at the site of inoculation rather definitely demonstrates the relation between the intensity of the local reaction and the protective mechanism invoked by it, as a reduction of the local lesion by unilateral castration led to an increase of the generalized lesions and the total suppression of the primary lesion by therapeutic agents also led to an increase in the general manifestations of the disease.[20]

The conclusions drawn from the experimental work in this connection were that the reaction at the site of inoculation tends to dominate the entire course of the infection, either inhibiting or obviating the necessity for the development of lesions elsewhere; and, conversely, that reduction or suppression of this local reaction by any means which does not also exercise an equal effect on the spirochetes removes this control and tends to increase the activity and severity of the infection and the occurrence of general lesions. These observations, while not in entire accord with those of Chesney, previously noted, cover a larger series of experiments over a longer period of observation and the conclusions drawn seem warranted by the data presented.

These experiments and conclusions have a very definite relation to the clinical management of syphilis as indicating that partial or subcurative treatment, by repressing the local reaction without coincidently eradicating all foci of spirochetes, may directly contribute to more severe generalized types of the disease and there are reports of clinical experiences in conformity with this conception.

This has been experimentally demonstrated in rabbits which, under these circumstances, have even been shown to be more susceptible to reinfection than normal animals.[21]

These and other studies in the reaction to syphilitic infection, while far from entirely elucidating the mechanism concerned, are of definite value

and interest and warrant the hope that future work may lead to a clear understanding of the processes concerned with the consequent development of clinical methods of exactitude in treatment and control.

Not the least important of the deductions to be made from the work outlined is that variations in the degree and extent of infection in the experimental animals represent an orderly expression of the interaction between two sets of forces: "one directed toward the spirochete itself, the other whose object is the neutralization of toxic or harmful effects. These reactions proceed in parallel directions but not to an equal degree," for, while the defensive reaction may be carried to the point of complete neutralization of the power of the spirochetes to produce disease, infection is never thus entirely eradicated but merely remains latent.

"If one may regard a lesion as primarily an expression of toxic injury on the one hand and of a defensive or protective reaction on the other, an analysis of the phenomena of periodic development and resolution of individual lesions leads to the conclusion that there is a quantitative relation between the injury produced by the spirochetes and the reaction of the host; that when the injury has been checked, the reaction tends to decline; but as not all spirochetes are destroyed in this process, the cycle is repeated, the state of resistance mounting with each successive repetition until the surviving organisms are incapable of producing further injury."[22]

From these considerations have been developed the "law of inverse proportions" and the "law of progression or sequence."

The "law of inverse proportions" expresses the thought that the duration of any active manifestations of the disease, (lesion), is inversely proportional to the intensity and extent of the initial local reaction; and the expression of this law has been experimentally demonstrated by the experimental modification of the course and general characteristics of experimental syphilis almost at will by means of modifications in the degree and extent of the local reaction, subject to the second general principle expressed as the "law of progression."

The "law of progression" is based upon the fact that "when allowed to pursue an undisturbed course, syphilis tends to preserve an orderly progression with varying degrees of intensity and extent. This characteristic of the disease appears attributable to the fact that different groups of tissues are not equally adapted to the growth and multiplication of the spirochetes, on the one hand, and that they are not equally sensitive or reactive to the toxic effects of the organisms on the other. Moreover, there is a natural order of susceptibility and of involvement, and the protective influences arising from reactions taking place in one group of tissues is extended to others in an equally orderly fashion. Under normal circumstances, therefore, the sequence observed in the occurrence of manifestations of the disease may be regarded as an orderly progression whose direction is determined by the relative susceptibility of different tissue groups and whose limits are fixed by the sequence and extent of the defensive reactions."[22]

It is, of course, obvious that as the manner and method of attack are varied, so there will be corresponding variations in the degree and extent

of the defensive reactions, ''hence, it may be said that the manifestations of the disease presented in any given instance depend, not only on the general laws which govern syphilitic reactions, but also on a number of other circumstances which includes any and all conditions affecting the initiation of the infection, the resistance of the host, and the pathogenic properties of the organism themselves.''[22]

REFERENCES

[1]Brown, W. H., and Pearce, L.:   Penetration of the Normal Mucous Membrane of the Rabbit by Treponema Pallidum, Proc. Soc. Exper. Biol. and Med., 1921, xviii, 200.

[2]Brown, W. H., and Pearce, L.:   A Study of the Relation of Treponema Pallidum to Lymphoid Tissues in Experimental Syphilis, Jour. Exper. Med., Jan. 1, 1922, xxxv, 39.

[3]Eberson, F.:   Dissemination of Spirocheta Pallida in Experimental Syphilis, Arch. Dermat. and Syph., February, 1921, iii, 111.

[4]Engman, M. F., and Eberson, F.:   An Experimental Study of the Latent Syphilitic as a Carrier, Jour. Am. Med. Assn., Jan. 15, 1921, lxxvi, 160.

[5]Brown, W. H., and Pearce, L.:   Latent Infections with Demonstration of the Spirocheta Pallida in Lymphoid Tissues of the Rabbit, Am. Jour. Syph., January, 1921, v, 1.

[6]Brown, W. H., and Pearce, L.:   Syphilitic Infection of the Central Nervous System of the Rabbit, Arch. Dermat. and Syph., November, 1920, ii, 635.

[7]Chesney, A. M., and Kemp, J. F.:   Experimental Observations on the ''Cure'' of Syphilis in the Rabbit with Arsphenamine, Jour. Exper. Med., April, 1924, xxxix, 4, p. 553.

[8]Brown, W. H., and Pearce, L.:   Superinfection with Treponema Pallidum, Proc. Soc. Exper. Biol. and Med., 1921, xviii, 200.

[9]Eberson, F.:   Immunity Studies in Experimental Syphilis.   Arch. Dermat. and Syph., June, 1921, iii, 775.

[10]Shinkle, C. E.:   Chancre in a Juvenile with General Paralysis.   Jour. Am. Med. Assn., May 31, 1924, lxxxii, 1780.

[11]Brown, W. H., and Pearce, L.:   Multiple Infections with Treponema Pallidum in the Rabbit.   Proc. Soc. Exper. Biol. and Med., 1921, xviii, 200.

[12]Eberson, F.:   Immunity Studies in Syphilis.   Arch. Dermat. and Syph., June, 1921, iii, 775.

[13]Eberson, F., and Engman, M. F.:   A Biologic Study of Syphilis: Latency a Biologic Reaction.   Arch. Dermat. and Syph., April, 1921, vii, 347.

[14]Chesney, A. M.:   Influence of Age, Sex, and Method of Inoculation on the Course of Experimental Syphilis.   Jour. Exper. Med., Nov. 1, 1923, xxxviii, 5, p. 627.

[15]Brown, W. H., and Pearce, L.:   The Reaction of Pregnant and Lactating Females to Inoculation with Treponema Pallidum, Am. Jour. Syph., October, 1920, iv, 4.

[16]Moore, J. E.:   Studies on the Influence of Pregnancy in Syphilis, Bull. Johns Hopkins Hosp., March, 1923, xxxiv, 89.

[17]Brown, W. H., and Pearce, L.:   Animal Resistance and the Endocrine System of the Rabbit in Experimental Syphilis, Proc. Soc. Exper. Biol. and Med., 1923, xx, 476.

[18]Brown, W. H., and Pearce, L.:   Neoplasia in Experimental Syphilis, Proc. Soc. Exper. Biol. and Med., 1921, xviii, 201.

[19]Brown, W. H., and Pearce, L.:   Experimental Production of Clinical Types of Syphilis in the Rabbit, Arch. Dermat. and Syph., March, 1921, iii, 254.

[20]Brown, W. H., and Pearce, L.:   The Resistance (or Immunity) Developed by the Reaction to Syphilitic Infection and Some of the Effects of the Suppression of this Reaction, Arch. Dermat. and Syph., December, 1920, ii, 675.

[21]Brown, W. H., and Pearce, L.:   Superinfection in Experimental Syphilis Following the Administration of Subcurative Doses of Arsphenamine or Neoarsphenamine, Jour. Exper. Med., May 1, 1921, xxxiii, 5, p. 553.

[22]Brown, W. H., and Pearce, L.:   The Defensive Reactions of Animals Infected with Spirocheta Pallida, Jour. Am. Med. Assn., November, 1921, lxxvii, 1619.

—R. A. K.

The American Society of Clinical Pathologists

Teamwork of the Surgeon and Pathologist

The American College of Surgeons recognizing the importance of the clinical laboratory on the scheme of hospital standaldization and the influence of the American Society of Clinical Pathologists in elevating the scientific and social status of the clinical pathologist has adopted the custom of inviting a representative of the A. S. C. P., at its national and sectional meetings.

Our secretary, Dr. Ward Burdick, read a paper before the Rocky Mountain Section of the A. S. C. P., which met at Pocatello, Idaho, last summer and delivered an address on the subject of "Financing the Hospital Laboratory" at the Clinical Congress of the American College of Surgeons, recently held in New York City. The surgeon, no less than the internist, appreciates the services of the clinical pathologist in helping him arrive at a diagnosis. The hospital laboratory has become a meeting place for the clinicians where they hold consultation with the pathologist on their cases with greater benefit to the patients. It makes an ideal arrangement, removing the impersonal element in laboratory work and introducing the human factor, at the same time directing the talents of both the clinician and the pathologist to the formulation of a correct diagnosis.

Membership in the A. S. C. P.

The remarkable achievement of the society in raising the status of the clinical pathologist from his former humble station as a technical aid to the eminent position of consultant has received due recognition from the best clinical pathologists of the country who have enrolled under its banner.

It is not the aim of the society to constitute an aristocratic or exclusive organization, and it therefore extends an invitation to medical graduates who are devoting all, or the major portion of their practice to clinical pathology to become members and participate in its activities. Further details as to qualifications may be had by corresponding with the secretary, Dr. Ward Burdick, 652 Metropolitan Building, Denver, Colo.

Standardization of Laboratories

The evolution of the clinical pathologist as a specialist has not followed along the same lines as his older confreres in the fields of surgery, laryngology or ophthalmology. It was taken for granted that the surgeon, the laryngologist or the oculist must be first of all a graduate physician and then have acquired the science and skill of his respective specialty. Little attention was paid to his instruments and armamentarium, not so, however, with the clinical pathologist. From the very genesis of this branch of medicine as a separate specialty, emphasis has been laid on his equipment, that is on the laboratory,

rather than on the intellectual and scientific endowment of its director. We are unfortunately wont to speak of the ''laboratory,'' probably as a figure of speech, when we actually mean the clinical pathologist. The result of this misapplication of words has produced the psychologic impression that the physical laboratory with its instruments and reagents is the important factor, while the one who carries out the determinations and interprets the findings is a secondary proposition.

It is not surprising, therefore, that early in the development of our specialty, abnormal manifestations began to appear. Instead of a healthy growth patterned after the evolution of the other fields of medicine, degenerative tendencies showed themselves, becoming plainly evident by a commercialization of this specialty. The outward signs were advertising through the mail and in medical journals and the formation of ''chain of stores'' laboratories. The general practitioner seeing these advertisements in respectable medical journals was unconsciously led to believe as it were, that the laboratory was a sort of contrivance where you placed a specimen in the hopper and received a diagnosis at the other end. The personal element was left out of account. Little wonder, then, that irresponsible laboratories, often conducted by incompetent lay technicians sprang up in the larger centers of population and by the usual methods of cut throat competition, fee splitting, contract practice and in general resorting to unethical measures, have produced a deplorable situation in some parts of the country resulting in great harm to patients and loss of prestige and usefulness of the ethical clinical pathologist.

The American Society of Clinical Pathologists, has from its very inception, led an active fight against the morbid manifestation in the development of this important field of medicine. Our members will recall the bulletins issued by our organization dealing with the advertising evil. As a result, the medical journals, while not altogether discontinuing laboratory advertisements, have entirely stopped the publication of fees. The cancer is, however, not all eradicated, and therefore, deserves the attention of all who are interested in scientific medicine. The American Medical Association has recognized the seriousness of the situation by appointing a special committee to look into this question. Evidence of the activity of this committee is shown in a questionnaire that is being sent to clinical pathologists throughout the country asking for data regarding their laboratories. While allowing full credit for the good intentions of the committee, the personnel of which has our highest respect, may we in all humility call attention to the fact that here too, the center of gravity seems to rest on the laboratory qua laboratory and its equipment. Data are requested for the name of the ''Institution,'' the housing, space occupied, dimension of rooms, equipment, etc. Questions are asked whether the laboratory does this or that kind of work? At the same time it is only fair to mention that there is a query as to the personnel: Is the director a medical graduate, his education, experience, duties, fraction of time spent in laboratory.

We do not desire to be captious or pedantic when we insist that the laboratory *per se* is secondary to the clinical pathologist. It is not mere word splitting. The American College of Surgeons when they inquire into the qualifications of an aspiring applicant for fellowship make no inventory of his surgical instruments or, similarly the American College of Physicians do not look into the office equipment of their applicants. The same is true with the ophthalmologist and laryngologist. It is the man behind the gun. We should hold the clinical pathologist responsible for his work which, of course, necessitates an adequate equipment. A mere collection of glistening bottles, a gaudy array of microscopes, microtomes, and colorimeters on glittering glass tables does not necessarily betoken exact and reliable findings and interpretations. One would rather be operated upon on a kitchen table by a skillful surgeon than in the best equipped operating room by a novice.

It is not our intention to put any impediments in the way of the good work of the committee, but rather to urge our members to fill out the data requested as their compilation will serve a most useful purpose. The questionnaire has but been a peg on which we could not resist hanging our pet thesis: It is the physician at the head who makes the laboratory.

CLINICAL AND EXPERIMENTAL

UNDERGRADUATE TEACHING OF CLINICAL PATHOLOGY IN THE MEDICAL SCHOOLS*

By Francis B. Johnson, M.D.,† Charleston, S. C.

THE object of this paper is to bring to your attention a subject in which the method of teaching has varied to a great degree in the undergraduate medical schools of this country. Its purpose will be amply fulfilled if it helps in any way to unify the teaching of clinical pathology or even points clearly to the need of this.

The importance of this to all of us, whether teachers or not, bears directly upon the development of clinical pathology as a division of medical practice and upon the recognition that will be accorded it by graduates of our medical schools. If the undergraduate medical student be thoroughly trained in the importance of the proper choice of methods and of accuracy of technic, and observes the relationship of the teaching clinical pathologist to the clinicians, he will when graduated recognize the practicing clinical pathologist as a consultant who is prepared to aid him in the study of his cases.

In the development of the modern school there was for a long time a sharp distinction between preclinical and clinical teaching. The preclinical courses were taught as pure sciences and given with little relationship to the patient. The clinical teachers were men of the older school, with little knowledge of the preclinical medical sciences as then taught. There was a gap between these two requiring to be bridged.

Within the past fifteen to twenty years, courses in clinical laboratory

diagnosis gradually evolved; starting usually as a course in urinalysis and adding to itself other chemical and microscopic examinations. This course now embraces a systematic study of the laboratory methods used in the examination of the urine, blood, sputum, gastrointestinal contents, exudates and transudates. The subjects are considered with special reference to their bearing upon the modern methods of microscopic, bacteriologic, serologic, and chemical technic used in diagnosis.

This course offered the first link in binding the preclinical to the clinical subjects for aid in diagnosis. For many reasons it could not bring all phases of the preclinical fundamental sciences in close relationship to the patient. On account of the content of the preclinical courses they have to be taken up in a scientific way and gone into more deeply than the clinician has time or opportunity to do. The necessity of still linking more closely the first two years to the other two has continued to be felt and efforts are now being made to introduce the clinical viewpoint more thoroughly into the fundamental sciences. There must be a certain and sure foundation in the study of the normal and of the causative factors of disease for the proper building, in order that the student can be developed to appreciate the necessity of correct methods and observations in his clinical work.

In order to get as accurate an idea as possible of the present status of clinical pathology in the medical school and the trend of opinion as to its function, its content and best mode of teaching, I have studied the catalogues and have sent questionnaires to all teachers of clinical pathology in the fifty-nine four year Class A Medical Schools. We have been pleased not only at receiving as high as eighty per cent of replies, but also by the great interest manifested in the results by those concerned. There are also included in the analysis thirty-four replies to a questionnaire sent by James C. Todd to prominent clinical teachers of medicine and surgery which he has very kindly turned over to me to use.

FUNCTION OF A COURSE IN CLINICAL PATHOLOGY

Before the student enters upon the course in clinical pathology he should have completed all the standard courses in the fundamental sciences with the possible exception of pathology which should, at least in part, have been covered.

Clinical pathology should not be a course in, or a renewal of these subjects except to perfect technic. The student should have an understanding of technic, observation of the normal, with some comparison to the abnormal, before arriving at clinical pathology to perfect himself in the clinical laboratory of the abnormal. Perfection of technic in the essential tests is the first principle to be taught. If accuracy is not assured in laboratory test and observation, interpretation loses its importance. We quote Dean Emerson (Indiana), as follows: "The essential function of a course in clinical pathology is training in accuracy * * * careless technic not only injures ward work, but what is more important, lowers the efficiency of the physician in all his future practice."

More specifically it seeks to train the student thoroughly in the technic

of such laboratory methods as he will have occasion to use in his practice, and to give him a general knowledge of the technic of those which are too complex for office use. It endeavors to teach the purpose of the procedures, to make clear their limitations and to correlate the findings with the underlying pathology and the clinical evidences of disease.

This instruction should be carried out not only during the introductory course, but during the remainder of the time the student is at college, by constant supervision of his laboratory work and conclusions in regard to ward and outpatient cases to which he is assigned.

In outlining our view of the function of clinical pathology, we do not wish to imply that it is a separate and independent part of the curriculum. Indeed no subject in the medical curriculum can be separate and independent. Clinical pathology is placed by most schools under the Department of Medicine, in some under the Department of Pathology and Bacteriology, in some, divided among several departments, while in others it is recognized as a separate department. No matter where placed, it cannot at all serve its proper function without the closest and most cordial cooperation with, not medicine alone, but all the purely clinical departments, both in its teaching, and in the laboratory work that it controls in the teaching hospital.

PLACE IN CURRICULUM AND EXTENT OF COURSE

When the course in clinical pathology was introduced some years ago in the medical curriculum, it was designated as "Clinical Microscopy" and was given in the fourth year. The name and place in the curriculum has been gradually changed, so that now the course is most frequently called "Clinical Pathology," though sometimes designated "Laboratory Diagnosis," or "Clinical Diagnosis," and is introduced at the time or just before the student starts his clinical work. At present, about one-half of the whole number of schools give the course in the third year, thirty-two per cent in the second year and eighteen per cent in a course extending through part of second and third years. In 1921 only fourteen per cent gave the course in the second year. This irregularity in the introduction of the course has presented some difficulties in the acceptance of credits when a student transfers from one school to another. From observation of the catalogues, over some years, it is evident that there is a continued tendency toward starting, if not completing, the formal course in the second year.

Based on information obtained, the number of hours for the formal course, exclusive of the laboratory work done in connection with hospital assignments in the third and fourth years, varies widely in the different schools from thirty-six to two hundred and fifty-six hours. Approximately one-third of the schools give from one hundred and twenty to two hundred and fifty-six hours. One-third give ninety to one hundred and twenty, one-third under ninety hours; and of these latter there are four that give less than fifty hours. The average number of hours was about one hundred.

This extreme variation emphasizes the need of some sort of standardization, if for no other reason than to facilitate transfer of students from one

institution to another. We believe these wide discrepancies do not represent the relative amount of laboratory diagnosis taught in the respective schools, but rather the extent to which clinical pathology is recognized as demanding a separate place in the curriculum.

No definite standard can be set, but if the course is to carry out its proper function, it is preposterous to expect to cover it thoroughly in thirty-six to forty-eight hours or even sixty hours. We do not wish to criticize the schools giving these brief courses for we realize that it is quite possible that adequate instruction in the subjects covered is given in related courses. The report of the committee on Undergraduate Teaching of Medicine of the Association of American Medical Colleges in 1921 recommended that one hundred and eighty hours be devoted to this subject.

VALUE OF THE DAILY CONCENTRATED COURSE

Pedagogically speaking this question is of much practical importance to both teacher and student. Of replies received to this question 82 per cent were opposed to this form of teaching. The usual reason stated was insufficient time to study and review ground covered in the laboratory. It is difficult to state exactly what is meant by "concentration"; this may be better understood by the term "block system." One, or possibly two courses are taken up, with the exclusion of all others, until completed. We feel that for acquiring accuracy of technic a certain degree of "concentration without isolation" is essential, i.e., laboratory periods of three hours in which are included thirty to forty minutes lecture or demonstration. We understand that in colleges of liberal arts where pedagogy is usually more considered than in medical schools, the ideal arrangement is three class periods a week. Students must have time to digest what they have learned, to reflect, and to compare.

From Herman (Michigan) we have the following reply: "The danger of a concentrated course seems to me to be that the students so often "cram" the information in, get 'by' the course and promptly forget all about it, * *"

CONTENT OF THE COURSE

Replies to the question, "Do you think that the required course should be limited to such ordinary routine laboratory examinations as a general practitioner would be expected to do in his office?" were diverse and interesting.

Opinion as to what should be considered ordinary routine laboratory examinations, no doubt, varies considerably. We see no reason why the general practicing physician, when time permits, should not include in his office work such laboratory examinations as the ordinary urinalyses, including microscopic examinations for blood counts and malaria, smears for gonococci, sputum for tubercle bacilli, feces for parasitic ova, test meals, and organisms in spinal fluid. The more of this work he does on his patients the better physician he will be.

Nearly two-thirds of the clinicians thought that the technical part of the course should cover only the ordinary routine that a physician would be

able to do in his office, while about three-fourths of the clinical pathologists replied that it should cover considerably more. Both classes agreed almost unanimously that by demonstration and lectures, a knowledge of the application of the more difficult methods should be obtained by the student, from both the clinical pathologist and the clinician.

Among those in favor of "ordinary routine" Sturgis (Harvard), referring to my question, states: "The above is the exact criterion I use in selecting material to be used in my course."

Of those answering that the course should consist of more than the ordinary examinations, we wish to quote the following:

Dean Emerson (Indiana) replies, "The student will never do as much laboratory work in general practice as he is taught to do in the school. Once during his medical course, therefore, he should be required to study his cases as nearly 100 per cent well as possible."

We agree with Vogel (Columbia) that, "Ordinary routine should be especially stressed, but other more advanced methods should be brought to the attention of the student." Our opinion is that he should be taught sufficient knowledge of the more refined methods of laboratory diagnosis to enable him to assimilate their relative value Unless considerably more than ordinary routine is included he will not be able to get a broad view of laboratory interpretation. The student should have a clear understanding of the relationship of laboratory tests to other signs and symptoms, a knowledge of the practical value of the results, and an appreciation of certain technical difficulties, not only of the ordinary simple methods, but of the more complicated procedures as well. He should also, by training, acquire sufficient knowledge to be able to read and understand current medical literature on modern laboratory diagnosis.

To ascertain more definitely which of the more complicated procedures should be made an essential part of the regular course, which merely demonstrated to the class, which made elective, and which considered sufficiently covered in other courses to be used clinically, we asked specifically about the status of ten of these procedures

It would be difficult to draw any very definite conclusions as the requirements and conditions varied considerably in the different schools. Even where subjects are mentioned as being taken up in clinical pathology, the extent could seldom be determined. The following summary is, however, of interest:

1. General cultural methods, isolation of bacteria, typing of pneumococcus, and animal inoculation are generally considered by a large majority of the replies to be well enough covered in the course in bacteriology. Blood cultures are, however, an exception to this, for more than half the schools give this as repeated in clinical pathology; in 40 per cent, as required, and in others as demonstrated or elective.

2. Diagnostic blood chemical examinations are assigned to clinical pathology by about three-fourths of the medical schools. Nearly one-half of the schools make it a required part of the course, and in the others it is demonstrated or elective.

3. Serodiagnosis (distinguished from principles of immunology) is assigned to clinical pathology by two-thirds of the replies. Most of this, with the exception of Widal tests, is given as elective. In certain schools, however, Wassermanns are placed as required or demonstrated.

4. The colloidal gold test, determination of basal metabolism and complicated functional tests are included as either demonstrated or elective in clinical pathology by three-fourths of the schools.

5. Skin tests, as tuberculin, Schick, and foreign protein-pollen sensitization, are mentioned as being given in clinical pathology in a few schools.

Most of the above subjects are given in their fundamental aspects in other departments, the clinical importance being demonstrated in clinical pathology. The opportunity for the student to perfect himself in technic and to continue the study of them clinically should be elective in the third and fourth years under supervision of the clinical pathologist. The promotion of this encourages a keen sense for investigation and we find our best students eager to follow when encouraged and led on. It is during this period that we take the first steps in the development of our future clinical pathologist, but of course it must not be carried too far. Vogel (Columbia) writes: "* * * we are not trying to make scientist and research workers, but doctors." However, we would reply to this that we must not lose sight of the fact that if we do not instill some desire for investigation and research, medicine will not become a science, but a lost art.

The questions, "Do you think the present medical student is taught so much dependence upon laboratory methods that he neglects the working out of other clinical evidences of the cases to which he is assigned?" and "Where would you say the fault lies?" bring us to a subject that was widely discussed.

The point is, not that the student is taught too much of laboratory methods, but whether he is taught too much dependence upon laboratory methods. The opinions of the clinicians and the pathologist were quite divergent upon these questions. About two-thirds of the clinicians agreed that the student is too dependent upon the laboratory and about two-thirds of the clinical pathologists maintained that he is not. The clinician has probably the better opportunity to judge.

Dean Graves (Louisville) writes as follows: "Any person with common sense who has enjoyed a well-balanced preparation in laboratory sciences and has applied these methods to clinical medicine is in no danger of neglecting either the laboratory or the clinical signs and symptoms."

On the other hand Dean Emerson (Indiana) writes: "Undoubtedly the student leans too heavily on laboratory methods, and still more heavily on the reports of laboratory work which others do. * * *" He later adds: "This does not mean that we should teach less clinical pathology; rather a great deal more."

Bentz (Buffalo) replies: "Too much of clinical systematology is taught without proper correlation."

Some of our respondents that deny that the student is too dependent on laboratory diagnosis offer, however, an opinion as to where the fault lies. We quote the following opinion of Cummer (Western Reserve): "Too much

emphasis placed on the laboratory by teachers of the fundamental sciences in the first two years, and lack of perspective by teachers of this subject. The worst offenders, however, are the old time clinicians who either scorn all laboratory tests, or go to the other extreme of placing too much emphasis on the findings.''

Keegan (Nebraska): ''This depends entirely upon the organization of the clinical staff. Ignorance on their part leads either to indiscriminate use of laboratory reports, or to a belittling attitude towards all laboratory work.''

Several of the clinicians agree with Christian (Harvard), who writes: ''In my judgment, a common fault of medical schools is the attempt to teach the student too many of the elaborate methods of examinations whether laboratory or otherwise, with the neglect of drill in the simple methods of medicine.''

Most of the replies agree that it depends upon the teachers both in the laboratories and the clinic. Students are just what we choose to make them. They can be made to take a keen interest in their work so long as the proper incentive is placed before them.

The chemist, the physiologist, the bacteriologist, and pathologist emphasize, and possibly overstress, certain points in their subjects in order that the student can more fully grasp and retain them.

The clinical pathologist, while he adds the clinical viewpoint, likewise brings forward the laboratory side of medicine. This all comes in part or entirely before the student arrives in close contact with the patients. There is much to consider in the force of first impressions.

We believe the student does depend too much at first upon his laboratory diagnosis. However, after two years in clinical work in which he is guided by the clinicians, with the cooperation of the clinical pathologist, he should have reached the point of determining the relative value of his laboratory results to the other signs and symptoms of disease. The student gets his first ideas of laboratory interpretation through the clinical pathologist and should continue to be under his supervision and receive his advice throughout his clinical years.

This is a debatable point in some schools and so the question was asked: Should interpretation of laboratory findings be taught by the clinical pathologist, by the internist, or by both?

Only two of the clinical pathologists and three of the clinicians stated that interpretation should be taught by the internist alone. The rest all agree that it should be taught by both. Sturgis (Harvard) writes: ''The greatest emphasis should be placed on technic in this course. A certain amount of interpretation should be taught, but the internist has the greatest opportunity to teach this.''

Kinsella (Washington) believes, ''The clinical pathologist should be an internist.'' According to our views he should at least make rounds in the teaching hospital frequently enough to keep in contact with interesting cases and not spend all his time merely teaching in his laboratory and doing mechanical work. In some schools he acts as a clinical teacher in the hospital wards, as well as instructor in the laboratory.

A clinical pathologist is not merely a technician; there is the necessity to keep up with the clinical side of medicine, otherwise he cannot teach his subject with the due consideration of interpretation that belongs to it. This is important, since it involves the whole question of the status of the clinical pathologist. We insist that as a teacher he is even less a technician than a clinical pathologist practicing his profession outside.

Both in his teaching and his capacity as consultant, he is concerned with the indications for certain tests, with the principles of interpretation and with the exact significance of the tests in particular cases. Undoubtedly, however, the final evaluation of the laboratory findings in relation to the data obtained from all other sources must rest with the clinician.

A PROPOSED UNDERGRADUATE COURSE IN CLINICAL PATHOLOGY

It would be presumptuous in us to set down in detail the ideal course. There can in fact be no single ideal course, however well worked out, to fit the diverse conditions to be met in the medical schools. We believe there should be sufficient comparable standardization for credit in other schools, which at present is not the case. We shall, however, present an outline of a course which we believe well suited for schools like ours in which there is a teaching hospital in close physical relation with the school buildings.

It is necessary to keep rigidly in mind that we are not trying to make clinical pathologists but general practitioners. As we would plan the arrangement, it would be divided into the following parts.

Part I. A systematic course of about ninety hours, fairly concentrated in the later part of the second year, in which the technic of the ordinary simple routine tests is to be perfected, and a general understanding obtained of their relation to clinical diagnosis. We should not only teach the positive value, but also the negative value and limitations of laboratory findings.

The object of such a course is so that the student can start in, well prepared for the usual required laboratory examinations of the cases to which he may be assigned in his third year. The content should be routine urinalysis, hemoglobin, blood counting, appearance of cells, blood grouping, malarial parasites, stomach contents, feces for blood, parasites and ova, sputum and purulent exudates., including spinal fluid, for cytology and pathogenic organisms.

Part II. A course of about ninety hours in the early part of the third year, extending over three or four months. This represents a study of important diseases in which the laboratory viewpoint will be thoroughly worked out, clinics being held upon selected cases, showing the indications for and limitations of certain tests. Students under supervision will carry out the more ordinary routine tests simultaneously, checking their work, and instructors with student assistance will demonstrate all other more complicated and possibly useful ones that may throw light on the clinical diagnosis, thereby presenting complete exhaustive laboratory studies of the important diseases.

Part III. During the later part of the third and the whole of the fourth year, under proper supervision of technic, and consultation in regard to in-

dications for and result of tests, the students carry out the ordinary laboratory examinations upon the cases to which they have been assigned in hospital and outpatient service. Time does not permit the average student to do more.

Part IV. Elective courses. To properly qualified students of the third and fourth years, elective courses covering more thoroughly the more complicated technical procedures should be offered. Only subjects of clinical value should be included.

The proper observation of the working out of the tests in concrete cases, with enough clinical material for comparison, is in our opinion the real criterion of a Class A course in clinical pathology.

In some schools it may be found advisable to give the whole of the systematic course in the second year; in others more hours may be given in the second than in the third year. Such changes could readily be made, depending upon the permissible allotted time and proper arrangement to other courses.

We believe, however, that there should be promoted a better standardization, so as to place more equitably the year given, hours required and content of course, in order that the status will be more definitely recognized and fixed in the medical curricula of all medical schools.

SUMMARY

There has been in the medical curriculum a need and consequent development of a course for the perfection of technic and interpretation of laboratory methods used in diagnosis. This is most frequently designated as Clinical Pathology, or Clinical Laboratory diagnosis.

From a survey of replies to questionnaires sent to teachers of clinical pathology, medicine and surgery, and from catalogues, the following information has been obtained:

1. Courses in histology, biochemistry, bacteriology, immunology, and part if not all, of general and special pathology, should be completed before clinical pathology is taken up.

2. The time at which the course is given by the majority of schools is the first part of the third year; next in number is the latter part of the second year; some give it in the latter part of the second and first part of the third, a few others throughout the whole third year. All schools advise a continuation of laboratory work, under supervision, on cases later assigned to the student

3. In hours, the courses vary in the different schools from 36 to 252 hours; about one-third from 120 to 252; one-third from 90 to 120, and one-third from 40 to 80. The average number of hours was 100. One hundred eighty hours was recommended by the Association of American Medical Colleges in 1921. Three-fourths are opposed to the daily concentrated course.

4. The course should cover more than the ordinary routine examinations that a general practitioner would be expected to use in his office, was the opinion of more than three-fourths of the replies of the clinical pathologist.

Most clinical teachers replied that only ordinary laboratory methods be taught together with the application of these and the more complicated methods.

5. There appears to be considerable difference of opinion as to what special subjects out of the ordinary should be included. Most frequently required are blood chemicals, Widals, and blood cultures; most frequently demonstrated are complement-fixation and colloidal gold and basal metabolic tests; most frequently elective are basal metabolic, blood chemicals, and serodiagnostic methods. Most of the subjects are given in their fundamental aspects in other departments, their clinical importance demonstrated in the laboratory of clinical pathology and at the bedside.

6. Two-thirds of the replies of the clinicians are of the opinion that the student depends too much upon the laboratory findings, and two-thirds of the clinical pathologists believe that he does not. There is a general opinion, where this fault exists, that some of the teachers in the preclinical subjects do not keep sufficiently in touch with the clinical side of medicine, also the fault of some of the clinicians, who do not have a clear understanding of the use and interpretation of certain tests. By some of the clinicians, it is thought that too great a proportion of time is devoted to the laboratory.

An outline of a course is presented showing place in curriculum, hours, and content which is believed to be suitable for schools in which there is, in close physical relationship, a teaching hospital.

A plea is extended for a better, more equitable standardization of the course and a more definitely fixed status of clinical pathology in the curricula of the medical schools.

CERVICAL SYMPATHECTOMY IN ANGINA PECTORIS*

With Report of a Case

By William H. Holmes, M.D.,† and S. W. Ranson, M.D.,‡ Chicago, Ill.

THE first attempt to relieve the pain of angina pectoris by removing portions of the left cervical sympathetic chain was made by Jonnesco[1] in 1916. Four years later when he presented his report, the patient had had no recurrence of pain. That this remarkable result did not attract more attention was probably due to the fact that Jonnesco had previously advocated cervical sympathectomy for other conditions such as hyperthyroidism, tic douloureux, epilepsy and glaucoma, and had claimed results which others subsequently were unable to obtain. The operation of cervical sympathectomy, therefore, was generally abandoned. Very recently, however, Coffey and Brown,[2] impressed by the report of Jonnesco's case of angina pectoris decided to sever the connections between the superior cervical sympathetic ganglion and the heart in a patient having frequent and severe anginal attacks. The operation was followed by complete relief from pain. Nine days later they performed the operation on a second patient with similar results. Their third and fourth patients were also relieved of pain but the fifth died six hours after operation. Brown,[3] one of the surgeons, advises us that at first they cut the main trunk of the left cervical sympathetic and the superior cardiac branch arising from the ganglion itself. Later on they removed the ganglion in order to be certain that all of its connections were cut. They have performed the operation twelve times, once on the right side in a case of dextral radiation. The attacks of substernal pain were stopped in every case but one, a syphilitic with aneurysm. In two cases a modified form of pain persisted in the region of the apex and left forearm.

The objective signs following removal of the ganglion are enophthalmos, myosis and pseudoptosis, narrowing of the palpebral aperture and absence of sweating and flushing on the affected side, constituting the syndrome of Horner. If the entire cervical sympathetic chain on one side is injured, the exact area in which sweating does not occur is bounded by a line which runs down the middle line of the head and neck and turns horizontally across the chest at the level of the third rib in front, and the spine of the scapula behind, including the whole of the upper limb.

In our own case, as in those of Coffey and Brown the paroxysmal pain of angina pectoris was relieved by removal of the superior sympathetic ganglion. The patient was a white woman, aged fifty-three years, who was

admitted to Wesley Memorial Hospital on January 29, 1923, for treatment of cardiac failure. For about one year prior to the appearance of edema, dyspnea, and other signs of decompensation, she had vague pains in the precordium and left arm. During the first part of her hospital residence she did not complain of pain. Her first attack of typical angina followed the effort of getting into a wheel chair. Thereafter, pain recurred with increasing frequency and severity until the slightest exertion was sufficient to bring it on. Nitroglycerin and amyl nitrite gave almost immediate relief but the subsequent prostration was extreme. On June 12, 1923, the superior cervical sympathetic ganglion was removed by William E. Shackleton and saved for histologic
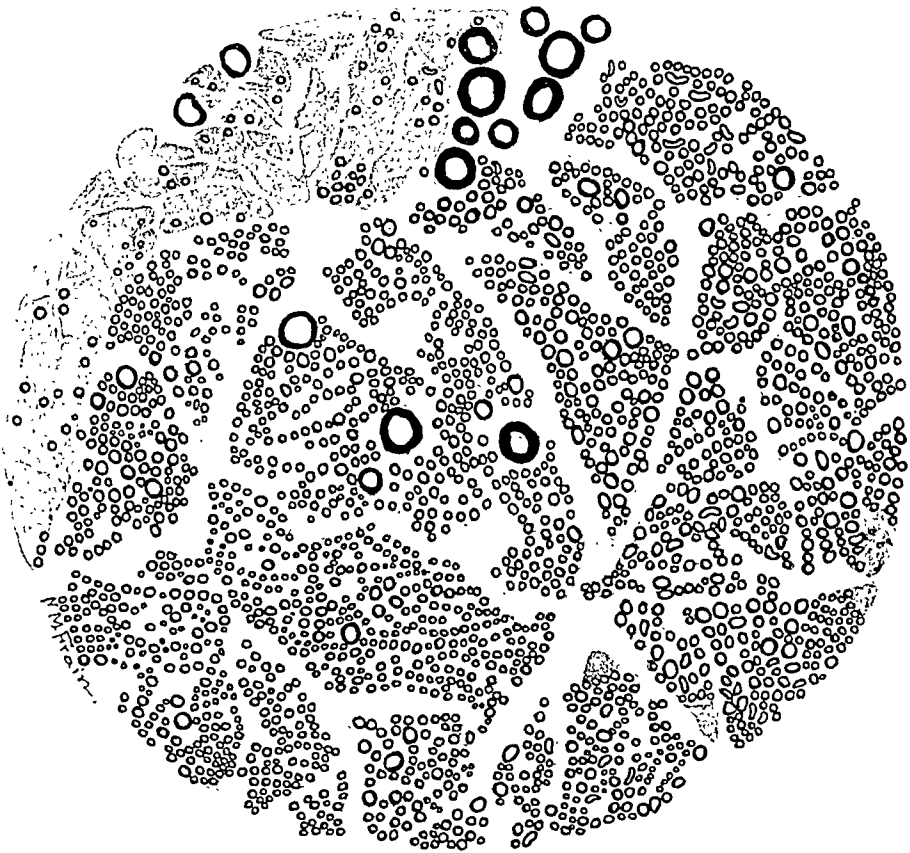
Fig. 1.

study. She was discharged from the hospital August 6, 1923, and soon thereafter was earning her living as a nurse in charge of two small children. At no time following the operation did she experience pain either in the thorax or left arm. Just a few days before the Christmas holidays she started on an automobile trip to Atlanta, Georgia. En route she became ill and was admitted to the Nashville General Hospital. A letter from the hospital dated December 29, 1923, states that she complained of severe abdominal pain, nausea, vomiting and of mild precordial pain just before her admission. The temperature, pulse, respiration, blood pressure, etc., were reported to be about normal. She recovered from this undiagnosed illness, was discharged from the hospital, and is now in Chicago. During the eleven and one-half months

she has remained under our observation, she has not had a single recurrence of thoracic or brachial pain of the anginal type.

Without entering into a lengthy discussion of the various theories which have been advanced in explanation of the symptomatology of angina pectoris it seems reasonable to assume that the structural changes are in themselves, insufficient to produce the paroxysmal symptoms observed in the ordinary case. In the extraordinary case in which the structural changes are extensive and of rapid development as in coronary thrombosis, pain is present in agonizing intensity but it is continuous, not paroxysmal. Allbutt believes the additional factor required to produce paroxysmal anginal pain is essentially a change in intraaortic pressure. Others think vascular spasm either of the aorta or coronary arteries is the cause of the pain.

Cerv Symp Trunk
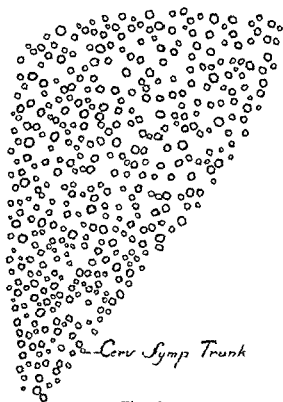
Fig. 2.

Surgical methods of treatment of angina pectoris have, heretofore, always been directed toward the relief of pain by severing the sensory fibers to the heart and aortic arch. This was accomplished by Jonnesco when he removed the lower cervical and first thoracic sympathetic ganglia, but at the same time he also destroyed efferent fibers to the aortic arch and coronary arteries. As shown by Coffey and Brown and confirmed by our own case the same beneficial results may be obtained simply by removal of the superior cervical ganglion, through which, according to the best information available, none of the cardiac sensory fibers pass. We would attribute the beneficial effect of high cervical sympathectomy to section of vasoconstrictor fibers, instead of to section of sensory fibers as is done by Coffey and Brown. It is important, therefore, to determine the course of the sensory fibers from the heart and the course of vasoconstrictor fibers going to the coronary arteries and aorta. The course of the cardiac sensory fibers is, we believe, fairly well established on the basis of histologic, physiologic and clinical evidence.

Histologically, it is possible to trace sensory fibers through the sympa-

thetic system because of their relatively large size. Edgeworth[4] has traced large sensory fibers to the cardiac plexus in the dog from the vagus nerve and from the white rami of the upper three thoracic nerves. Those from the white rami reached the cardiac plexus after passing through the stellate ganglion, the annulus of Vieussen, the inferior cervical ganglion and the nerve passing from that ganglion to the heart. One of us (S. W. R.)[5] by the study of serial sections of the sympathetic trunk in the cat has been able to confirm Edgeworth's findings. The large sensory fibers in the upper three thoracic white rami (of which the second is illustrated, Fig. 1) can be traced through the annulus of Vieussen and thence through the cardiac branches that spring from it, and from the inferior cervical ganglion. No large sensory fibers were found in the cervical sympathetic trunk going to the superior ganglion. The nerves removed from our case of angina on histologic examination showed no large sensory fibers in either the cervical sympathetic trunk or the superior
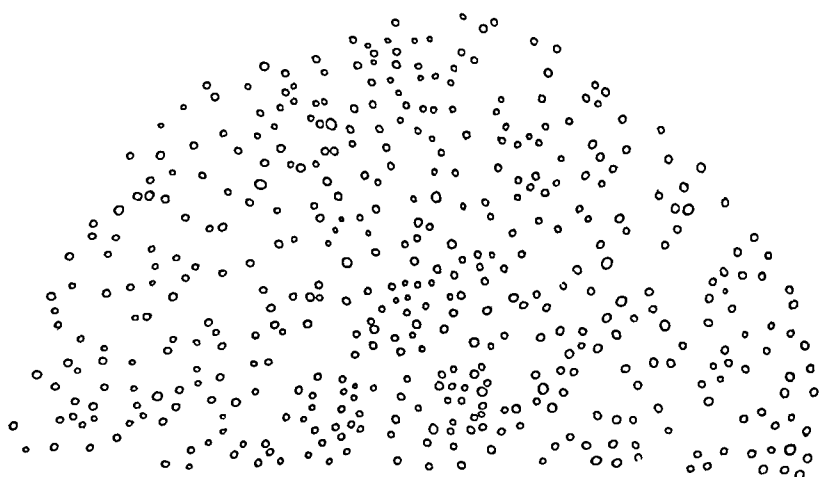
Sup. Card. Nerve

Fig. 3.

cardiac nerve which descends from the superior cervical ganglion to the cardiac plexus. (Figs. 2 and 3.) There is, therefore, no reason for assuming that the sensory pathways for the heart in man are essentially different from those in the cat and dog. The course of the sensory fibers from the heart as determined by histologic studies is shown in the diagram. The arrows indicate the direction of conduction and the dotted line a possible but highly improbable pathway. (Fig. 4.)

This conception of the sensory pathways is supported by physiologic and clinical evidence. Langley, using electrical stimulation, could find no evidence of sensory pathways in the cervical sympathetic trunk. Head has shown that the thoracic and brachial pain in angina pectoris is referred along the distribution of the three upper thoracic nerves of the left side. This demonstrates that this type of pain is mediated by the white rami of these three nerves. It excludes the possibility that it is mediated either through the vagus or through hypothetical connections of the superior cervical ganglion with the brain. In this connnection it will be remembered that Wencke-

bach in his address before this Society last year claimed to have stopped anginal attacks by cutting the depressor nerve. While this nerve is recognized as a distinct branch of the vagus in the rabbit, its fibers are incorporated with the vagus in man. Granting that it may have a separate course for a short distance as Odermatt[c] and some other European workers claim, it is scarcely conceivable that pain which is referred to the chest wall and left arm, should be relieved by section of any part of the vagus nerve. We cannot help wondering if in these operations the superior cardiac nerve which runs through the operative field has not been damaged. Nor can the relief
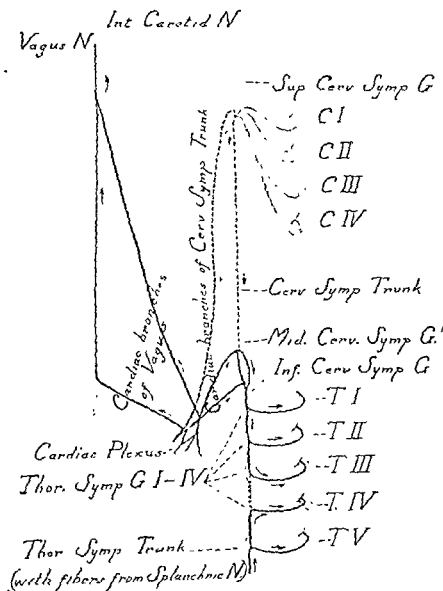
Fig. 1

given by high cervical sympathectomy be due to the interruption of sensory paths through the thoracic white rami, because these nerves are not even exposed to operative trauma in removing the superior cervical ganglion. That the sensory pathways through the thoracic white rami are not destroyed by resection of the superior cervical ganglion is also indicated by the fact that this operation does not invariably stop referred pain of cardiac origin. The continued pain in Coffey and Brown's case of syphilitic aneurysm in spite of the removal of the superior sympathetic ganglion may have been due to direct pressure on the vertebrae or sternum, but this would not explain the presence of pain in the forearm in two of their uncomplicated cases of

angina. The persistence of pain in the forearm proves the presence of intact sensory pathways through thoracic white rami to the corresponding segments of the spinal cord.

That cervical sympathectomy does not relieve pain merely by interrupting sensory pathways is also indicated by the fact that in successful cases patients are often returned to a life of usefulness. The sense of constriction, the fear, the physical prostration and other evidences of angina pectoris were in our case relieved quite as much as was the pain. From being bed-ridden because of anginal attacks brought on both by effort and emotion, the patient, after the operation, was able to follow a gainful occupation requiring considerable effort. Cervical sympathectomy, therefore, apparently brings about a cessation of paroxysmal pain not by cutting sensory pathways but by interrupting an efferent mechanism. It must be admitted that we know nothing about such a mechanism, but it is not unreasonable to suppose that vasoconstrictor fibers, reaching the heart through the superior cardiac nerve, may be at fault. This nerve is not present in the dog but its place is perhaps taken by fibers which pass through a branch of communication to the vagus and along that nerve to the heart. Wiggers[7] has shown that stimulation of the vagus nerve on either side causes constriction of the coronary arteries. Since these arteries contract when perfused with physiologic salt solution containing adrenalin, it is probable that the vasoconstrictor fibers found by Wiggers in the dog's vagus have their origin in the superior cervical sympathetic ganglion. The superior cardiac nerve is not present in animals and consequently is not subject to direct experimental investigation. In the human we know that it takes part in the formation of the cardiac plexus but in the adult this plexus is so intricate as to defy analysis.

The embryologic picture, however, gives us a clue. In the human embryo the superior cardiac nerve goes to the bulbar plexus which surrounds the bulbus cordis and its derivatives the aorta and pulmonary artery while the middle and inferior cardiac nerves go to the atrial plexus. The atrial plexus, since it supplies the region in which the heart beat originates may be assumed to receive the sympathetic cardio-accelerator fibers. The bulbar plexus is situated near the beginning of the aorta. From it arise offshoots forming plexuses on the coronary arteries. These offshoots may be assumed to receive vasoconstrictor fibers which probably come through the superior cardiac nerve. Since the aorta is formed from the left side of the divided bulbus cordis it might be assumed that the vasoconstrictor fibers for it come from the left superior cardiac nerve. If future investigation should prove that this assumption is correct it would be possible to explain the effects of high left cervical sympathectomy as due to the interruption of the vasoconstrictor path to the aorta and coronary vessels.

The idea that the paroxysms of angina pectoris are due to structural change plus vascular spasm has long held a prominent place among the theories advanced in explanation of the symptoms of the disease. Not being able to account for the results obtained by section of the superior cardiac nerve on the basis that it contains sensory pathways we advance the hy-

pothesis that this nerve contains vasoconstrictor fibers for the aorta and coronary arteries and that section prevents spasmodic vasoconstriction and, therefore, stops paroxysmal pain, but is without effect on pain caused entirely by structural changes.

REFERENCES

[1]Jonnesco: Bull. de l'Acad. de méd., Paris, October, 1920, lxxxiv, 93. Presse méd., 1921, xxix, 193.
[2]Coffey, Walter B. and Brown, Philip King: Arch. Int. Med., February, 1923, xxxi, 200.
[3]Personal communication January 10, 1924.
[4]Edgeworth, F. H.: Journal of Physiology, 1892, xiii. 260.
[5]Ranson, S. W.: Jour. Comp. Neurol., 1918, xxix, 313.
[6]Odermatt, W.: Deutsch. Ztschr. f. Chir., 1923, clxxxii, 341.
[7]Wiggers, C. J.: Am. Jour. Physiol., 1909, xxiv, 391.

ON THE ALLEGED SYNERGISM OF MAGNESIUM SULPHATE AND MORPHINE WHEN INJECTED PRIOR TO THE INDUCTION OF ANESTHESIA BY THE ETHER-OIL COLONIC METHOD[*]

By Harry Beckman, M.D., Milwaukee, Wis.

IN 1921 Gwathmey,[1] reviewing the work of Meltzer and Auer and others by which the anesthetic properties of magnesium sulphate were established, concluded "that magnesium sulphate is a safe agent in producing anesthesia" and that "when used synergistically, it is absolutely harmless and one of the most effective agents of which we know." He states that the synergistic effect of magnesium sulphate upon morphine was suggested to him by Pellini and that working together they determined the effect of this combination, as follows: "A sufficient number of animal experiments was conducted to prove that ⅛ grain of morphine in 2 c.c. of a 25 per cent solution of chemically pure and sterilized magnesium sulphate, given hypodermically and repeated twice at half-hour intervals, analgized an animal sufficiently for the full force of an artery clamp to be placed anywhere on the skin without being noticed by the subject. The controlled animal, with the same dosage of morphine given in plain water hypodermically, was not analgized to anything like this degree." This is adduced as evidence of the effectiveness of the combination, nothing being brought forward to prove the "absolutely harmless" nature of the same—indeed, no mention is made of the relative state of depression of these animals either during or following the experiments. As further evidence in favor of the combination, the statement is made that "at the Presbyterian Hospital it has been definitely determined that the addition of a small amount of magnesium sulphate to the usual hypodermic of morphine increases the value of the hypodermic from 50 to 100 per cent."

Gwathmey therefore proposed the conversion of colonic anesthesia, as previously developed by him, into synergistic analgesia by making use of the

synergistic effects of magnesium sulphate and morphine when the two drugs are injected hypodermically prior to the introduction into the colon of the ether-oil mixture.

Since the appearance of the paper referred to, Weston and Howard[2] have reported the giving of from two to six c.c. of a 50 per cent magnesium sulphate sterile solution, subcutaneously and intramuscularly, in more than 1000 instances in the insane in states of more or less excitement and insomnia. In 82.7 per cent of the cases the sedative action was prompt, the patient becoming quiet after fifteen to thirty minutes and sleeping from five to seven hours. In a few instances a patient became quiet but did not sleep. "The effect persisted for from five to ten hours." They have also used the salt in a number of persons, not insane, but suffering from severe pain, with very gratifying results. They failed to find any evidence in favor of the alleged synergism of magnesium sulphate and morphine, for they found "that in cases not quieted by six to 10 c.c. of 50 per cent magnesium sulphate solution, morphine was also of no value except in heroic doses, and that when the magnesium sulphate alone failed to give the relief it was of no effect when used in combination with ¼ grain of morphine."

Recently Gwathmey, Donovan, O'Reagan, and Cowan[3] have published a preliminary report of clinical experiments having for their object the production of painless childbirth by synergistic methods, one of the drugs advocated being magnesium sulphate. They state that with the synergistic method they attempt to secure the relaxation with the magnesium sulphate as well as using it for its power of prolonging the effect of the morphine. In this report they are concerned chiefly with the relief of pain and the condition of the baby.

In view of the paucity of experimental data upon which to base a belief in the synergism of magnesium sulphate and morphine, the writer has undertaken a study of the question by performing a definite number of carefully controlled experiments.

The work has been projected upon the following schema, dogs being used as subjects in all cases:

(a) A series of trials in which morphine alone is to be injected before inducing anesthesia by the ether-oil colonic method; the object here is the determination of the exact amount of ether which can always be counted upon to induce and maintain surgical anesthesia.

(b) A series of trials in which both magnesium sulphate and morphine are to be injected before inducing anesthesia by the ether-oil colonic method, using an amount of ether less than that determined in (a) to be always effective. The object here is twofold: First, to determine whether the amount of ether can be radically reduced because of the synergistic effect of the magnesium sulphate upon the morphine—in other words whether ether-oil anesthesia can be converted into ether-oil analgesia, due to the action of the magnesium sulphate. Second, to observe any deleterious symptoms which might arise subsequently to the injection of the magnesium sulphate, and which might rightly be attributed to the same.

(c) A series of trials in which calcium salts are to be injected at such time, and in such manner, as best to overcome any deleterious effects of the magnesium which might be observed in (b). The object here would be to lessen the toxicity of the magnesium without decreasing too much its synergistic effect upon the morphine.

Series (a) and (b) of these observations will be reported upon in this paper.

<div align="center">SERIES (A)</div>

Method.—Dogs, having been starved for eighteen hours, were injected subcutaneously in the side with an aqueous solution of 0.01 gm. morphine sulphate per kilo body weight. After one hour they were tied to a dog-board upon their left side; a No 29 soft rubber catheter was lubricated and introduced slowly into the rectum and colon for an average distance of seven inches, and the ether-oil mixture allowed to flow in during four minutes. Gwathmey[4] having suggested 75 per cent ether and 25 per cent olive oil, a mixture in these proportions was used in all cases, with the following results:

(1) One ounce of the mixture per 40 lbs. of dog was ineffective in inducing anesthesia.

(2) One ounce of the mixture per 30 lbs of dog was little if any more effective.

(3) One ounce of the mixture per 20 pounds of dog was in all cases effective in inducing and maintaining surgical anesthesia. Ether was usually detected on the breath before the last of it had been introduced into the colon, anesthesia being complete in an average time of thirteen minutes. There were no failures. In a few cases there was some slight loss due to bubbling of gas up through the tube, or around it at the anus; but estimate of the amount lost was made and then that amount added. Complete surgical anesthesia lasted from one and a quarter to one and a half hours; in two cases, at the first signs of returning consciousness, another full dose of the ether-oil mixture (one ounce per 20 lbs.) was introduced and anesthesia maintained for an additional hour and a half. The usual test for complete anesthesia was the ability to make an abdominal incision through all the layers of the skin in a dog having complete muscular relaxation. However, in order to be doubly sure, in three cases (selected at random) the abdomen was completely laid open and rough manipulative procedures were performed upon the gut. Muscular relaxation was always complete, though the partial retention of reflexes was always noted; in two cases dogs responded to a whistle by partially raising the head while major surgery was being done on the abdomen.

Careful observation of the respirations and pulse were made in all cases, and the absence of salivation and of any movements of the body in the second (excitement) stage remarked in all cases.

A single attempt to use the method without preliminary morphine failed; the dog fought fiercely, and the very small amount of the mixture which was successfully introduced served only to produce a violent excitement stage which lasted for some time after the dog was released.

In this series eight dogs were used, some of them being anesthetized as often as five times at intervals of one week. Altogether the method was used twenty-four times. A protocol, selected at random from the series, is here given:

Dog B.—March 3. Male, 14 kilos, estimated age 1 yr. Condition good, 3rd anesthetic.

12:00 M. —Morph. Sulph. subcut., right side, 0.14 gm. (3.5 c.c. of a 4 per cent solution.)

1:08 P.M.—Placed on board, tied down on left side.

Resisted pricking of skin of abdomen with knife.

Respiration 18, pulse 84.

1:14 to 1:21 P.M.—Rectal tube introduced (very little resistance) and 47 c.c. of ether-oil (75% E, 25% O) introduced (1 oz. per 20 lbs.).

1:26 P.M.—Dog cannot be aroused but eyes are open.

Lid reflex undiminished.

1:30 P.M.—Respiration 20, pulse 104.

Untied and placed on back. Muscular relaxation complete, eyes half closed.

Lid reflex present but diminished.

1:35 P.M.—Incision made down to peritoneum. Anesthesia perfect.

Three interrupted sutures, painted with iodine and alcohol;

Respiration 24, pulse 116.

1:40 P.M.—Anesthesia continues. Respiration 24, pulse 120.

1:45 P.M.—Anesthesia continues. Respiration 24, pulse 120.

1:50 P.M.—Anesthesia continues. Respiration 26, pulse 116.

2:00 P.M.—Anesthesia continues. Respiration 26, pulse 122.

2:10 P.M.—Anesthesia continues. Respiration 28, pulse 122.

2:20 P.M.—Anesthesia continues. Respiration 28, pulse 120.

Full force of hemostat on belly wall; no response.

2:25 P.M.—Introduced small catheter beside the other and began washing out.

2:31 P.M.—Washing out completed, 500 c.c. tap water. Returning water oily with slight odor of ether.

2:36 P.M.—Moving head about and pawing with front legs.

2:52 P.M.—Rolled himself off the dog board.

3:02 P.M.—Dog returned to kennel; moves about on legs groggily, selecting place to lie down; lies down in natural position.

4:00 P.M.—Sleeping but quite easily aroused.

March 4.

9:00 A.M.—Dog completely recovered.

In none of the dogs has there been any symptoms of colitis, or of even the very slightest rectal irritation. One of the dogs fell sick after having been anesthetized (colonic) five times at intervals of one week. Symptoms: malaise, weakness of front legs, temperature 103.2° F. This dog was then given the usual dose of morphine but anesthetized by the inhalation method, 25 c.c. of ether being required by the open drop method to establish the surgical stage. The animal was used for routine laboratory work and at the end of two and one-half hours, when killed, a total of 161 c.c. of ether had been used; the amount required in the colonic method in this length of time would have been 86 c.c. (115 c.c. of the mixture). The colon and rectum were carefully examined and no evidence whatever of inflammation therein could be found. This dog had several wounds upon the abdomen and probably died from septicemia; the other organs were not examined.

In the interval of one hour, or a little more, between the injection of the morphine and of the ether-oil mixture, the dogs were allowed their freedom

in a large space. During this time they defecated and urinated several times, but vomited only rarely. They feebly resisted catching, and the weakness of the hind legs, typical of morphine, was observed in all cases. When placed upon the anesthetizing table they showed no signs of respiratory embarrassment, though the amplitude of the respirations was perhaps slightly less than in the normal and the rate a little slower. In all cases they were able to stand, though somewhat groggily, one-half hour after the ether-oil mixture had been washed out of the colon, and when removed to the kennels at this time they would always move about and select their own place for lying down. They were usually removed to the kennels between four and five o'clock in the evening, were always found to be completely recovered at nine in the morning, and remained in a perfectly normal state thereafter.

SERIES (B)

Method.—Dogs, having been starved for eighteen hours, were injected subcutaneously in the side with an aqueous solution of 0.01 gm. morphine sulphate per kilo body weight (same amount as in Series (a)), and at the same time, either in that or the other side, magnesium sulphate was injected, 2 c.c. of a 50 per cent solution being given in all cases. The site of the magnesium sulphate injection was massaged for about one-half minute. After one hour they were tied to the dog board and the catheter introduced, but the amount of the ether-oil was reduced to one ounce per 40 lbs., an amount which had always been ineffective in inducing anesthesia when morphine alone had been previously injected.

With the same criteria for surgical anesthesia as in Series (a), it cannot be said that anesthesia was ever successfully induced in this magnesium sulphate series. The dogs were brought to the table in apparent distress in all cases; they were whining; the rate of the respirations was not reduced more noticeably than when morphine alone was used, but the respirations were jerky and there was almost an expiratory grunt; and in most cases there was a quite profuse salivation. A number of these dogs have pawed at their nose, a symptom which is taken by many observers to be a sign of respiratory embarrassment. The jerky respirations have persisted throughout the attempt to induce anesthesia, and several times the moaning has persisted also. Though the dogs have usually seemed to lose consciousness, they were never completely relaxed, and always resisted deep cutting. They fail to return rapidly to consciousness after being washed out and are much depressed for usually two days after.

In this series five dogs were used, some of them as often as four times at intervals of one week. All of the dogs had previously been successfully anesthetized in Series (a). Altogether the method was used twelve times. A protocol, selected at random from the series, is here given:

Dog E.—April 7. Male, 9 kilos, estimated age 1½ yr. Condition good. Two anesthetics in Series (a).

12:00 M. .—Morph. Sulph. subcut., right side, 0.09 gm. (2.25 c.c. of a 4 per cent solution).
            Mag. Sulph. subcut. right side, 1 0 gm. (2 c.c. of a 50 per cent solution). Massaged ½ min.

1:04 P.M.—Placed on board, tied down on one side. Dog moaning constantly.
Respiration 24, jerky. Pulse 108. Marked salivation.

1:08 P.M. to 1:16 P.M.—Tube inserted, 16 c.c. of ether-oil (75 per cent E., 25 per cent O)
introduced. (1 oz. per 40 lbs.).

1:20 P.M.—Respiration 26, jerky. Pulse 110.

1:24 P.M.—Moaning ceased, resp. continued jerky.

1:30 P.M.—Dog placed on back but rolls himself on to left side again.

1:35 P.M.—Resp. 26, jerky. Pulse 110.

1:40 P.M.—Dog placed on back and remains there but is not completely relaxed.
Attempt to incise abdomen is resisted by movements of legs and· moaning is
recommenced.

1:50 P.M.—Respiration 28, jerky. Pulse 118.
Stops moaning.
Attempt to incise unsuccessful as before; moaning does not recommence.

2:02 P.M.—Attempt to incise resisted. No moaning.

2:05 P.M.—Catheter introduced and washing out begun.

2:12 P.M.—Washing out completed, 500 c.c. tap water. Returning water oily with slight
odor of ether.

2:30 P.M.—Has made several voluntary movements of legs but is still lying on back on
the board.

2:50 P.M.—Removed to kennel. Very limp, lies in unnatural position in which placed.
Resp. continues jerky.

4:00 P.M.—Dog has changed position but very little. Awake and moaning. Respiration
continues jerky.

April 8 and 9.
Dog appears sick. Lies on side, or wanders around kennel as if in a stupor.
Moans for long periods. On April 9, at 4 P.M., expirations still partake some-
what of their former jerky character.

April 10.
Dog appears normal.

A typical picture in this series on the first, and to a less extent on the
second day after the injection of the magnesium sulphate, is of a very sick
looking dog lying flat on the side with legs extended, moaning, and with
jerky expirations; this will alternate with periods of standing in a corner
with drooped head, with moaning, and jerky expirations, stupid expression,
and an occasional lurching forward to keep the body from falling. The
salivation persists only for a few hours after injection of the salt. A large
abscess has appeared at the site of the magnesium injection in five of the
twelve cases, whereas no abscesses have occurred from the morphine injec-
tions in either of the series. The pus evacuated has not been cultured, but
is odorless. The solutions used have not been sterilized.

In two cases, outside this series, 4 c.c. of the magnesium sulphate were
given, the other conditions being the same as when only 2 c.c. were given.
In one of these cases the anesthesia was complete, which was proved by
performing mutilating operations in the abdomen; the dog was of course
killed at the end of the experiment. In the other case the anesthesia was
never perfect, and the depression after the dog was washed out was so great
that calcium chloride was given to save the dog; though as the calcium chlo-
ride was injected subcutaneously (4.5 c.c. of a 50 per cent solution), it is not
certain that it was an effective agent in promoting this recovery.

DISCUSSION

Series (a).—So far as a parallel may be drawn between dog and human work, all the advantages claimed by Gwathmey[5] for ether-oil colonic anesthesia are amply borne out by these experiments. These advantages can perhaps best be pointed out by making use of Gwathmey's original headings:

"*Safety.*"—While the induction of third stage anesthesia with ether in an average time of thirteen minutes, by placing within the body at one time the entire amount of ether to be used, may appear somewhat dangerously precipitate, it is in fact only apparently so. The rate at which ether separates from ether-oil mixtures *in vitro* was shown by Baskerville[6] in 1915 to be a definite and fairly fixed one. This definite rate of separation must apply also under the conditions obtaining *in vivo;* for were it possible for all, or a large part, of this ether to become available for absorption into the circulation at any one time, it should be expected that at least some cases of too profound anesthesia, if not actual deaths, would have been encountered in the series. No such cases occurred; but on the other hand, in all instances a definite quantity of ether per pound body weight produced a satisfactory, but not too deep anesthesia, which lasted for a predictable length of time, after which the animal quickly regained consciousness. This is taken as evidence that only a small portion of the total amount of ether placed in the colon is available to the circulation in a unit of time. The partial retension of reflexes (the lid reflex has never entirely disappeared) must be taken as further evidence for safety; indeed some of the animals seemed not entirely to have lost consciousness, for in two cases feeble response was made to a whistle while major surgery was being performed upon the abdomen.

"*Comfort.*"—The animal is not subjected to the indignity of being forcibly held while the objectionable cone is placed over the muzzle, nor does he make even the slightest struggle during the second stage.

"*Control.*"—When the correct amount of the mixture is administered per pound body weight, the method is absolutely controllable in both directions: the anesthesia may be terminated at any time by washing out the colon with tap water, with a rapid return of consciousness; the state may be prolonged for any desired length of time by adding, for every additional hour of anesthesia required, one ounce of the mixture per 20 lbs. body weight. If, at any time, the anesthesia seems too light, a cloth may be placed over the muzzle, thus compelling a partial rebreathing of the fumes, with an appreciable deepening of the state.

"*Efficiency.*"—Whenever one ounce of the mixture per 20 lbs. is used perfect anesthesia results. The actual amount of ether used—22.5 c.c., 75 per cent of the mixture—for inducing and maintaining surgical anesthesia is much smaller than required in any other method available to us for safe work in the laboratory.

"*Simplicity.*"—The apparatus required is one No. 29 soft rubber catheter, one No. 13 same, a spring or screw clamp for each, two small funnels, and a receptacle to catch the oily water when washing out the colon. One introduces

the large catheter, which has previously been filled with the mixture so that it just shows in the attached funnel, for about 7 inches, and then slowly (during 4 minutes) pours in the measured quantity of the ether-oil, closing the clamp when the fluid just shows again in the funnel. The catheter is allowed to remain in place; the writer has usually tied it to the tail with a bit of string. At the conclusion of the experiment, the small catheter is introduced beside the larger one, the latter withdrawn somewhat, and the animal is washed out, introducing the water through the small catheter. The articles are always clean and never objectionable to use, an advantage which cannot always be claimed for the foul, saliva-soaked ether cones commonly employed in laboratories.

"*After-effects.*"—Symptoms of colitis have never been encountered. Recovery has always been prompt and lasting. A measure of analgesia seems to persist for some time, as the animals in this series have seemed startlingly unaware of their wounds several hours after regaining consciousness.

Series (b).—To the first of the questions investigated in this series, namely, does magnesium sulphate exert a synergistic effect upon morphine sulphate when injected prior to the induction of ether-oil colonic anesthesia, it is felt that a positive answer cannot be given at this time. I certainly failed to obtain any evidence in favor of this synergism when I gave the amount of magnesium sulphate (2 c.c. of a 50 per cent solution) which is said always to increase the value of the morphine from 50 to 100 per cent. However, I feel that the reduction in the amount of ether attempted may have been too radical, since Gwathmey,[1] in speaking of the reduction of the amount of ether to one-half in the human, states that 2 c.c. of a 25 per cent magnesium sulphate solution was given three times at intervals of one-half hour, the total amount of the salt injected thus being one-third more than was used by me. Therefore, I feel that the only statement warranted at this time is that under the conditions obtaining in these experiments no evidence in favor of the alleged synergism was obtained.

To the second of the questions investigated, namely, are there any deleterious symptoms arising subsequently to the injection of the magnesium sulphate which may rightly be attributed to the same, a positive answer can be given. In all cases in which the salt was injected in these experiments such symptoms did arise. I believe that a careful study of the classical experiments by which the anesthetic properties of magnesium sulphate were established would lead us to expect such symptoms, even when such small doses are used as those advocated clinically and used in these experiments. 1. Meltzer and Auer[7] showed conclusively (working with rabbits) that magnesium salts in intravenous injections are very toxic, even in small doses; the effect being upon the respiration, which becomes completely inhibited. 2. Intraspinally in monkeys,[8] magnesium sulphate caused the death of two of the six animals. They also state here that the relaxing effects may last twenty-four hours or longer. 3. Subcutaneously in rabbits,[9] 8 c.c. of a 25 per cent solution produced anesthesia from which the animals recovered; no recorded observations of their condition on subsequent days. Twelve c.c. of the same

solution was fatal. 4. Subcutaneously in cats,[9] the safe range was quite narrow: 0.8 gm. of the salt per kilo was unsatisfactory, 0.9 gm. produced anesthesia with recovery (no subsequent observations recorded), 1.0 gm. was fatal. 5. Subcutaneously in dogs,[9] 2.0 gm. of the salt per kilo, or a little more, was required to produce anesthesia; but the anesthesia would come on slowly, gradually become more profound, and finally lead to death. No animal survived, though they were all in a satisfactory state of anesthesia for some time before the terminal depression. When the injection was given intramuscularly, or part intramuscularly and part subcutaneously, somewhat more satisfactory results were obtained in that some of the animals survived. A state of depression on subsequent days is not remarked, except that in one case cited, the dog had lost nearly 1 kilo in weight after five days. 6. Subcutaneously in guinea pigs,[9] doses between 1.0 and 1.25 gm. per kilo were effective with recovery; 1.8 gm. per kilo caused rapid death 7. Subcutaneously in white rats,[9] the dose required to produce anesthesia—1.75 gm. per kilo—resulted in death half an hour after injection in three of the five cases.

Justification for the use of this highly toxic salt subcutaneously in the human is found, so far as the writer can discover, in the following reasoning: the fatal dose for the rabbit being x, the fatal dose for a man weighing y times as much as the rabbit, would be xy. This dose being determined, a small fraction of it is then injected subcutaneously in man as an "absolutely harmless" dose. This small dose—2 c.c. of a 50 per cent solution—when injected with morphine, prior to induction of ether-oil colonic anesthesia in dogs, has now been determined to be provocative of symptoms which, if not absolutely dangerous, are certainly highly undesirable.

CONCLUSIONS

1. One ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 20 lbs. body weight, introduced into the colon of dogs one hour after the subcutaneous injection of $\frac{1}{4}$ c.c. of a 4 per cent solution of morphine sulphate per kilo body weight, is effective in inducing safe and very satisfactory surgical anesthesia and in maintaining same for a little more than one hour.

2. One ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 40 lbs. body weight, introduced into the colon of dogs one hour after the subcutaneous injection of $\frac{1}{4}$ c.c. of a 4 per cent solution of morphine sulphate per kilo body weight and 2 c.c. of a 50 per cent solution of magnesium sulphate, is not effective in inducing satisfactory surgical anesthesia.

3. The subcutaneous injection of 2 c.c. of a 50 per cent solution of magnesium sulphate at the same time that $\frac{1}{4}$ c.c. of a 4 per cent morphine sulphate solution per kilo body weight is injected in dogs, when these injections are followed after one hour by the introduction into the colon of one ounce of a mixture of 75 per cent ether and 25 per cent olive oil per 40 lbs. body weight, gives rise to undesirable respiratory symptoms and a general depression, which usually lasts two days. These symptoms are directly referable

to the magnesium sulphate, because they do not arise when the salt is omitted, other conditions being the same.

These studies will be continued. The doses of morphine sulphate, magnesium sulphate and ether will be shifted about in many different combinations in an attempt to find some evidence in favor of the alleged synergism of magnesium sulphate and morphine. Calcium will also be used in an attempt to overcome the undesirable effects of the magnesium sulphate without lessening any synergistic effect it may be found to have upon morphine.

I wish to extend my hearty thanks to James T. Gwathmey, whose suggestions have been of the greatest help in planning these experiments.

REFERENCES

[1]Gwathmey, James T.: Jour. Am. Med. Assn., 1921, lxxvi, 222.
[2]Weston, P. G. and Howard, M. Q.: Am. Jour. Med. Sc., 1923, clxv, 3, 431.
[3]Gwathmey, James T., Donovan, E. P., O'Reagan, J. and Cowan, L. R.: Am. Jour. of Obst. and Gynec., 1923, vi, 4.
[4]Personal communication to the writer.
[5]Gwathmey, James T.: Am. Yearbook of Anaes. and Analges., 1915.
[6]Baskerville, Charles: Proc. Amer. Philosoph. Soc., liv, August, 1915, No. 218.
[7]Meltzer, S. J. and Auer, John: Am. Jour. Physiol., 1906, xv, 387.
[8]Meltzer, S. J. and Auer, John: Jour. Exp. Med., 1906, viii, 6.
[9]Meltzer, S. J. and Auer, John: Am. Jour. Physiol., 1905, xiv, 366.

CHEMICAL BLOOD ANALYSIS IN ASTHMA, HAY-FEVER AND ALLIED CONDITIONS*

By Maximilian A. Ramirez, M.D., A. V. St. George, M.D.,
and R. L. Moses, A.B., New York

THE study of the various immunologic phenomena underlying anaphylaxis and allergy have been extensively investigated and reported; but it is only within very recent times that attention has been focused upon metabolic changes occurring in these conditions. Most of these studies, however, have been confined to animals. Thus Hisanobu noted increases in the nonprotein nitrogen and urea in the blood obtained from guinea pigs following anaphylactic shock. Hirsch and Williams found a diminished alkalinity of the blood in guinea pigs while in anaphylactic shock. In some of these animals the reaction was of such a magnitude as to be incompatible with life. A disturbance in the sugar metabolism, following the injection of protein and peptone, was noted by both Kuryama and Major and subsequently, Pentamelli demonstrated a marked disturbance in the nitrogen metabolism in rabbits following the injection of horse serum and milk respectively. Rackemann, Longcope and Peters, in a series of ten postpneumonic serum disease sufferers, noted a marked, transient chloride and water retention.

There is evidence accumulating that alterations in the physicochemical status of cellular activity is a controlling factor in some immunologic phenomena. Whether this is due to merely an altered hydrogen-ion concentration, a colloidal cellular change, or an increase or diminution in protoplasmic irritability are problems which require solution. It is with the object of contributing to the literature on this subject that we are presenting a series of chemical findings obtained during the routine examination and treatment of forty patients in our clinic.

Unfortunately we have only recently commenced the determination of the mineral constituents of the blood and hence have not as yet collected a large enough series for publication. Nevertheless, especially the calcium content of the blood in true bronchial asthma and in bronchospasm, promises to be of interest.

The nonprotein nitrogen sugar and plasma chlorides were determined on all patients when first seen at the clinic and again at the conclusion of treatment.† While some investigators might deem uric acid and creatinine determinations of value, we have not obtained any figures permitting of interpretation and hence we have discarded them. The nonprotein nitrogen and

TABLE I

	NAME	SEX	AGE	DISEASE	BEFORE TREATMENT			AFTER TREATMENT			KIND OF TREATMENT	DURATION OF TREATMENT	RESULTS OF TREATMENT
					NPN	SUGAR	CHLO-RIDES C.C.	NPN	SUGAR	CHLO-RIDES			
					MG. PER 100 C.C.								
1.	J.A.	M	37	Asthma	36	102	640	34	110	510	Vaccine	16 months	improved
2.	C.M.	F	29	Urticaria	42	100	580	40	100	550	Food protein and diet	10 "	cured
3.	H.V.S.	M	64	Asthma	52	105	580	54	110	550	Horse dandruff	8 "	cured
4.	C.S.H.	M	39	Asthma	48	90	510	45	100	500	Vaccine	12 "	improved
5.	M.G.	M	48	Asthma	40	100	580	40	95	500	Vaccine	8 "	improved
6.	A.W.	M	36	Asthma	32	110	570	35	100	480	Vaccine	10 "	improved
7.	J.W.	F	28	Urticaria	36	100	590	35	100	540	Food protein and diet	6 "	not improved
8.	S.M.E.	M	29	Urticaria	40	105	540	40	100	500	Food protein and diet	4 "	improved
9.	J.H.H.	M	14	Hay-fever	28	100	520	34	95	500	Pollens	4 "	cured
10.	M.W.	M	26	Hay-fever	36	100	550	35	100	550	Vaccine and pollens	18 "	improved
11.	A.T.	F	28	Asthma	38	95	680	40	100	575	Vaccine	14 "	not improved
12.	C.R.S.	M	28	Urticaria	36	100	590	35	100	500	Food protein and diet	6 "	improved
13.	H.B.	F	29	Hay-fever	34	92	600	32	100	550	Pollens	4 "	improved
14.	M.K.	M	32	Asthma and Hay-fever	38	105	540	35	100	490	Pollens and Vaccine	18 "	cured
15.	H.O'B.	M	34	Asthma	40	110	550	40	100	500	Vaccine	13 "	improved
16.	K.S.	M	46	Urticaria	42	100	610	45	95	530	Food protein and diet	6 "	improved
17.	J.McM.	M	42	Hay-fever	39	105	550	40	100	540	Pollens	4 "	improved
18.	W.J.	M	26	Asthma	40	100	640	40	92	600	Vaccine	11 "	not improved
19.	E.M.	F	39	Eczema	32	105	590	30	100	510	Diet only	6 "	cured

TABLE I—(Cont'd.)

NAME	SEX	AGE	DISEASE	BEFORE TREATMENT (MG. PER 100 C.C.)			AFTER TREATMENT			KIND OF TREATMENT	DURATION OF TREATMENT	RESULTS OF TREATMENT
				NFN	SUGAR	CHLORIDES C.C.	NPN	SUGAR	CHLORIDES			
20. J.J.	M	28	Eczema	30	98	680	30	100	660	Diet only	8 months	cured
21. H.P.	F	32	Eczema	38	100	650	38	100	660	Diet only	3 "	not improved
22. J.M.	F	27	Eczema	35	110	650	40	100	600	Diet only	8 "	improved
23. K.	F	38	Asthma	40	80	668	45	80	608	Horse serum	14 "	improved
24. A.	M	24	Urticaria	80	182	650	49	122	563	Diet only	12 "	improved
25. T.	M	51	Hay-fever	40	84	600	35	102	640	Pollens	12 "	improved
26. S.	F	57	Asthma	39	96	640	37	87	388	Pollens	12 "	not improved
27. H.	F	38	Asthma	56.3	108	635	44	90	664	Vaccine	2 "	improved
28. F.	F	28	Asthma	38	90	611	46	88	619	Horse dander	6 "	improved
29. E.K.	F	42	Asthma	47	82	567	33	108	612	Vaccine	3 "	improved
30. R.E.	F	58	Hay-fever	35	80	625	38	82	603	Vaccine	2 "	improved
31. L.C.	M	16	Asthma	50	94	663	47	85	588	Vaccine	8 "	improved
32. D.S.	M	21	Hay-fever	33	80	631	38	100	588	Pollens	6 weeks	improved
33. F.J.	M	16	Urticaria	32	92	625	33	85	625	Diet and Peptone injection	3 months	cured
34. M.H.	F	48	Asthma and Hay-fever / Urticaria and Asthma	34	116	625	39	80	625	Pollens	9 "	not improved
35. C.C.	F	32	Hay-fever	41	90	650	50	90	618	Diet and Peptone injection	6 "	not improved
36. F.H.	M	29	Hay-fever	43	72	650	36	116	588	Pollens and Horse serum	2 "	improved
37. L.H.	M	34	Urticaria	35	90	644	32	80	619	Diet and Peptone	3 "	improved
38. A.S.	M	39	Asthma	40	92	650	40	90	637	Vaccine	4 "	cured
39. E.H.	M	51	Asthma	40	83	675	32	80	613	Vaccine	6 "	improved
40. T.B.	M	42	Hay-fever	42	76	650	35	82	600	Pollens	2 "	improved

sugar determinations were made in accordance with Folin's technic; the plasma chlorides according to the method of Friend, as modified by Hearn.*

As the standard average of these three constituents we have taken the following figures to represent the limits of normal.

Nonprotein nitrogen	25- 40 mg. per 100 c.c.
Sugar	80-125 mg. per 100 c.c.
Plasma Chlorides	550-600 mg. per 100 c.c.

These case records were selected, not on the basis of chemical reports, but on the clinical results—clinical cures—which were obtained. The patients have been free from symptoms at least six months and at least half of them were free of symptoms twelve to eighteen months from the cessation of treatment. The result of treatment was indicated as cured, improved and not improved.

Conclusions.—Nonprotein nitrogen, sugar and plasma chlorides were determined upon forty patients with asthma, hay-fever, and allied conditions. Determinations were made before instituting treatment and after completion of the same.

The resulting figures suggest that in asthma, urticaria, hay-fever, etc., there is a relative high plasma chloride content of the blood and to a less extent in the sugar and nonprotein nitrogen; the latter two especially in the nonallergic type of asthma cases with a long standing associated bronchitis.

It is impossible to state definitely that treatment caused a marked change in the chemical constituents of the blood, though a glance at the tables shows some diminution in the constituents after treatment.

REFERENCES

Hisanobu: Am. Jour. Physiol., 1919, 1, 357.
Hirsch and Williams: Jour. Inf. Dis., 1922, xxx, 259.
Kuryama: Jour. Biol. Chem., 1917, xxix, 127.
Major: Deutsch. Arch. f. klin. Med.. 1914, cxvi, 248.
Pentamelli: Pediatria, 1921, xxix, 481.
Rackemann, Longcope and Peters: Arch. Int. Med., 1916, xiii, 496.
Folin: Jour. Biol. Chem., 1919, xxxviii, 81 and 107.
Friend: Jour. Biol. Chem., 1922, li, 115.

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*The modification consists merely in the substitution of a standard solution of silver nitrate, of which 1 c.c. is equivalent to 1 mg. of sodium chloride, for the normal solution used by Friend.

A NOTE ON THE USE OF HEPARIN IN BLOOD TRANSFUSION*

By Edward C. Mason, M.D., Ph.D., Detroit, Mich.

INTRODUCTION

THE observations of Howell and Holt, which have been subsequently confirmed by the author, would lead one to conclude that heparin is an anticoagulant of first choice, it having no undesirable side reactions. Therefore in a previous study, based upon the results obtained from animals, it was recommended that heparin be considered as a clinical agent to inhibit intravascular coagulation. Before such clinical application could be made it was necessary to (1) develop a sterile product, (2) determine the toxicity for the human, (3) determine the dose necessary to give the desired delay in coagulation and (4) the length of duration of its action.

PROCEDURE

Sterilization.—The problem of sterilization of such a stable substance appeared to be quite a simple matter. However, in reality some difficulty was experienced in developing a sterile product without loss of potency. For clinical use it was recommended that heparin be put up in 1 c.c. ampules each containing 100 mg., but in the course of sterilization it was found more convenient to use 2 c.c. of solvent. Of the various methods of sterilization which have been developed, the choice for the present work has been to use 100 mg. of heparin dissolved in 2 c c of physiologic salt solution saturated with boric acid and 0.5 per cent chloretone.

The Determination of the Toxicity for the Human.—Prior to the use of the ampules in the human they were tested on several animals. Conditions were maintained analogous to those met with in transfusions except that the animals were either under ether or the effects of morphine. The apparatus used was that designed by Hartman. Such an apparatus furnishes a complete mixture of blood and anticoagulant soon after the blood leaves the vessel. The contents of the ampules were diluted with sterile physiologic saline to desired volume within the inner chamber of the apparatus.

The pharmacologic action of such preparations appeared to be quite analogous to that of the untreated heparin, there being no marked change in pulse, rate or volume, nor was there any noticeable change in respiration. For example: 150 c.c. of blood was drawn from a dog weighing 11.8 kilos, and the heparin solution was added at a constant rate. The total amount of heparin added was 32 milligrams which gave a concentration of approximately 1 milligram of heparin to 5 c.c. of blood. After ten minutes the blood was injected into the same animal and observations made. The animal appeared

perfectly normal after the operation, and at no time subsequently were there any symptoms suggesting discomfort. The dose in this case was 1.23 milligrams per pound of body weight.

After seventeen days the animal just described was used again but the procedure was reversed, the anticoagulant being injected into the circulation before the blood was withdrawn. The dose of heparin selected was 200 milligrams on the assumption that an animal weighing 11.8 kilos should have approximately one liter of blood. Five minutes after the injection 125 c.c. of blood was withdrawn. The shed blood was kept for about five minutes and injected back into the animal's circulation. A sample of the shed blood was kept and found to be fluid 24 hours later. Again the animal showed no
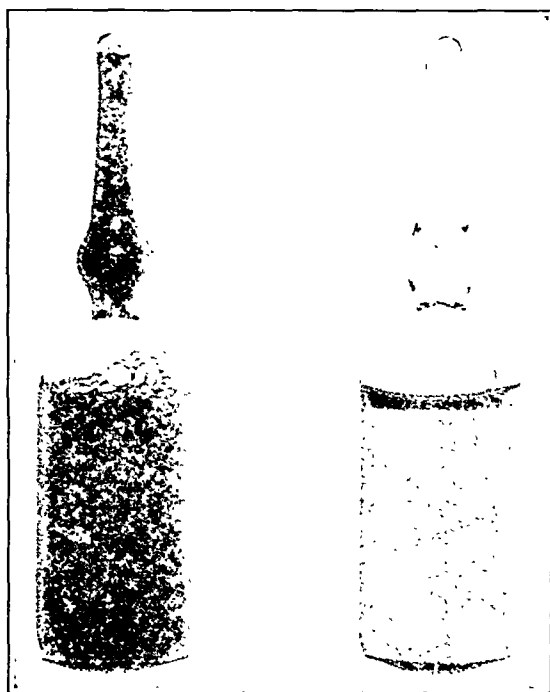
Fig. 1.

ill effects from the operation although the dose of heparin was 7.69 milligrams per pound of body weight.

The work was extended to observations on the human, and to date the anticoagulant has been used on 33 subjects in doses ranging from 50 to 300 milligrams. The dose most frequently used was 100 milligrams as that amount will prevent the coagulation of 500 c.c. of blood for 24 hours and therefore allows a safe margin for transfusions much above 500 c.c.

The use of heparin in the human gave results quite analogous to those observed in the animal studies except that often about 30 to 40 minutes after administration the patient developed symptoms which varied in intensity from mild headache and chilly sensation to intense headache, backache, vomiting, chills and temperature as high as 104.4 degrees. The cause of such untoward reactions is unknown. That it is due either to the anticoagulant or some substance associated with the anticoagulant is evidenced by the fact that the

reactions are experienced if the heparin be injected without the addition of blood, and, since different preparations vary greatly in their toxicity without markedly varying in their potency, it is suggested that the toxicity is due to an associated substance. Tests* have shown the material to be negative to the ninhydrin, biuret, Millons and acetic ferrocyanide tests for protein. The order of reaction is also against a protein reaction since patients often show a reaction with the first injection but not with subsequent injections. That the preservative is not the cause of the reaction is supported by observations with other preservatives (chloroform with evaporation at ice-box temperature) in which reactions were equally as common; also there are other preparations on the market in which saturated boric acid and .5 per cent chlorotone are used as preservatives, from which no reactions are experienced.

TABLE I

THE AMOUNT OF DELAY IN COAGULATION AND THE RETURN TO NORMAL

PATIENT	AMT. OF HEPARIN GIVEN	AMT. OF BLOOD GIVEN	AMT. OF BLOOD WITHDRAWN	COAGULATION BEFORE	COAGULATION AFTER	1 HOUR AFTER	1 HR. 30 MIN. AFTER	2 HOURS AFTER
No. 1	100 mg.	....	....	10 min.	33 min.	....	17 min.	....
No. 3	100 mg.	600 c.c.	....	8 5 min.	35 min.	....	....	Normal
No. 4	100 mg.	600 c.c.	....	7.0 min.	16 min.	....	7 min.	....
No. 12	100 mg.	500 c.c.	....	10 5 min.	....	14 min.	....	....
No. 18	200 mg.	600 c.c.	....	8 min.	....	12 min.	....	....
No. 19	200 mg.	....	150 c.c.	20 min.	60 min.	....	....	....
No. 26	100 mg.	....	350 c.c.	8 min.	41 min.	....	....	....
No. 33	75 mg.	....	....	11 min.	35 min.	22 min.	....	....

In the course of this study three different lots of ampules were used. The first gave reactions in 33⅓ per cent of the cases, the second gave 100 per cent reactions and the third gave 28 per cent reactions which for the most part were mild. The third lot of ampules represented a marked improvement in physical appearance as well as being the least toxic. Fig. 1 is presented to show the marked decrease in color as well as an absence of surface foam. Such a product has resulted through a slight modification in the mode of preparation.

The Delay Produced in Coagulation and the Time Necessary for Coagulation to Return to Normal.—In the use of such a potent anticoagulant for blood transfusions it is important that one know its effect on the coagulation of the recipient's blood. Therefore observations were made in which the recipient's coagulation time was determined before transfusion, immediately after transfusion and at varying periods following transfusion. Table I

*Made in the laboratory of Hynson, Westcott and Dunning.

shows briefly (1) the amount of heparin given, (2) the coagulation time before administration and after administration; (3) also the amount of blood given with the heparin or the amount of blood withdrawn from the subject, whichever the procedure may have been. The results rather closely agree with those obtained from animal observations in that the return to normal coagulation requires about 1 hour and 45 minutes to 2 hours.

Although our experience is quite limited there appears, from the cases studied, no marked increase in risk from hemorrhage following doses up to 100 milligrams. In one case markedly jaundiced there was actually an improvement in coagulation. Before receiving 500 c.c. of blood containing 100 milligrams of heparin the patient's blood showed a poorly formed clot in 20 minutes, and 30 minutes following the transfusion a good clot formed in 18 minutes.

CONCLUSIONS

1. Heparin as used in the early part of the work proved too toxic to warrant its routine clinical use in doses of 100 milligrams or above.

2. Subsequent preparations have given encouraging results, and it appears that with more work on the subject heparin may prove a desirable anticoagulant for clinical use.

REFERENCES

[1]Howell and Holt: Am. Jour. Physiol., 1918, xlvii, 328.
[2]Mason: Jour. Surg. Gyn. and Ob., 1924, xxxix, 421.
[3]Hartman: Jour. Am. Med. Assn., 1922, lxxviii, 15.

THE USE OF DEFIBRINATED BLOOD PRESERVED WITH FORMALDEHYDE FOR THE PURPOSE OF CONTROLLING DILUTIONS OF DRIED BLOOD*

By Ruth Gilbert, M.D., and Ellsworth A. Parsons, Albany, N. Y.

FOR several years, the problem of providing a satisfactory standard with which to compare dilutions of dried blood submitted for agglutination tests has presented considerable difficulty. Although, in this laboratory, the number of specimens received in the tube outfits is increasing, the majority of specimens are still submitted on slides. It is impracticable to estimate the amount of blood contained in such specimens, and therefore the color of dilutions made from them must be compared with that of dilutions which are known to be exact.

Only one method of diluting dried blood accurately has been found in a review of the literature. This is the method of Ruediger and Hulbert[1]

who suggest air-drying the blood for twenty-four ·hours and then weighing, using 0.1 c.c. of salt solution per milligram of blood for a 1:25 dilution. The impracticability of this method for routine work is obvious.

Heretofore, in this laboratory, known dilutions of dried human blood have been used in preparing color standards. Dried blood, however, usually assumes a brownish tint after forty-eight hours. Also, at times, parts of the specimen may peel off the slides.

The preparation of accurate dilutions of fresh blood each day is inconvenient, and it was therefore, considered advisable to investigate the use of defibrinated blood. Accordingly, a small amount of sheep's blood was defibrinated by shaking it in a bottle containing sterile glass beads for five or ten minutes, and to 3 c.c. of this, 0.15 c.c. of a 1·20 dilution of formalin (40 per cent formaldehyde) was added. The first specimen prepared remained satisfactory for a period of four weeks.

For purposes of confirmation and comparison, twelve different specimens of defibrinated sheep's blood were obtained on different days and treated with formalin. They were then diluted every other day for a period of two weeks. During that time, all were found to compare satisfactorily with accurate dilutions of freshly drawn human blood.

The dilutions are prepared in the concavities of a porcelain plate of the type used for diluting the specimens. Forty-five hundredths of a cubic centimeter of 0.5 per cent salt solution are pipetted into the first concavity, 0·03 c.c. into the second, and 0.09 c.c. into the third. Then, by adding 0.05 c.c. of the defibrinated sheep's blood to the first concavity, a dilution of 1:10 is obtained. Three hundredths of a cubic centimeter of the 1:10 dilution added to the second and third concavities gives a 1:20 and a 1:40 dilution respectively.

The specimen of dried blood is emulsified on the slide in a small amount of salt solution and transferred to one of the first concavities in a porcelain plate. More salt solution is then added if necessary until the color approximates as nearly as possible that of the 1·10 dilution of the defibrinated sheep's blood. The 1:20 and 1:40 dilutions are then prepared in the second and third concavities and the specimen is ready to be tested.

This method has been employed in the routine work and has proved to be a simple procedure for preparing color standards for use when specimens of dried blood are diluted for the agglutination test.

REFERENCE

[1]Ruediger, G. F., and Hulbert, R.: Is Dried Blood as Reliable as Fresh in Making the Widal Test? Am. Jour Pub. Health, 1914, iv, 113.

HISTOPATHOLOGIC AND BLOOD STUDIES WITH SILVER-SALVARSAN*

BY C. W. HOOPER, M.D., AND C. N. MYERS, PH.D., NEW YORK CITY

THIS is the third of a series of articles dealing with the retention and elimination of silver after the administration of silver compounds. In the first article of the series, Myers, 1923, carefully reviewed the literature, presented the reported cases of argyria in chronologic order and discussed the nature of the silver products that had been found to be the causative agents. The second article, by Myers and Corbitt, 1924, was an experimental study based upon the results obtained with 21 rabbits and approximately 220 rats. They compared the retention and elimination of the silver contained in silver-salvarsan with that contained in argyrol and cargentos. They found that after single massive intravenous injections of 200 milligrams silver-salvarsan per kilogram (equivalent to a single clinical dose of 12 grams) the albino rat excretes 96.5 per cent of the silver injected within six days. In comparable experiments in which they employed argyrol and cargentos it was found that about 20 times as much silver is retained on the sixth day in the case of argyrol, and 15 times in the case of cargentos as in silver-salvarsan. They also injected 21 rabbits with repeated doses of silver-salvarsan. The 17 rabbits that received 66.7 milligrams per kilogram repeatedly, excreted on the average over 90 per cent of the total amount of silver injected within a period of seven days after the last injection. The four rabbits that received 10 milligrams per kilogram excreted approximately all of the silver injected within the same period.

The purpose of this article is to describe the histopathologic changes found at necropsy in 20 of the rabbits and to present the blood counts and hemoglobin figures taken at weekly intervals during the entire course of the injections in eight of the rabbits.

EXPERIMENTAL

Twenty rabbits were employed as experimental animals. The silver-salvarsan was administered by injection from a syringe into one of the ear veins. One or two per cent solutions of the drug in freshly distilled water were used for the injections. In a few of the experiments 0.4 per cent sodium chloride was added. Sixteen of the rabbits received a dose of 66.7 milligrams per kilogram repeated at intervals of three to seven days. Four of the rabbits received 10 milligrams per kilogram repeated at the same intervals. The minimal amount of the drug injected was 223 milligrams in a period of 47 days and the maximal amount 2,363 milligrams in 70 days. The silver-

salvarsans employed, controls SS9 and SS11, were commercial preparations which had passed the official standard for toxicity. The observed arsenic contents were 21.0 and 19.31 per cent, respectively, and the silver contents were 14.5 and 14.3 per cent, respectively. After an interval of 6 to 44 days after the last injection the animals were sacrificed with chloroform and immediately necropsied. Three of the animals died within four days after the last injection of the drug from acute bronchopneumonia. Small pieces of the following organs were saved for section: cerebrum, bone marrow, lungs, heart, stomach, small intestine, spleen, adrenals and kidneys.

HISTOLOGIC EXAMINATION OF TISSUES

For histologic detail, the tissues were fixed in Zenker-formalin fluid, washed thoroughly in running water, hardened in alcohol, cut in paraffin, and sections stained with hematoxylin and eosin. The term Zenker-formalin fluid is used to indicate Zenker's fluid without the acetic acid, to which 10 per cent of neutral 4 per cent formaldehyde solution is added immediately before use.

For the microchemical demonstration and differentiation of silver, sections were first submerged in a saturated solution of iodine in alcohol for five minutes to dissolve any mercuric oxide crystals. They were then treated with a warmed aqueous solution of hydrogen sulphide for ten minutes to darken the silver granules and finally stained with hematoxylin and eosin. Other sections were treated with a strong aqueous solution of potassium cyanide for ten minutes to dissolve the silver granules.

Perl's reaction was employed to eliminate the possibility of iron. The sections were subjected for 10 minutes to a mixture of a 2 per cent solution of potassium ferrocyanide, one part, and a 1 per cent solution of hydrochloric acid, three parts, heated to 60° C, after which the sections were differentiated in 0.5 per cent hydrochloric acid solution and stained with neutral red and eosin.

BLOOD EXAMINATIONS

The rabbits were fasted sixteen to twenty hours immediately before the blood examinations. The blood was obtained from a freely bleeding ear puncture. The silver-salvarsan was administered uniformly after the blood had been obtained for the counts.

The hemoglobin determinations were made by means of the Sahli hemoglobinometer. The color tube of the hemoglobinometer was standardized against blood having an oxygen capacity of 18.5 volumes per cent by the Van Slyke gasometric method, 1918 (approximately 14 grams hemoglobin per 100 c.c.).

The red and white cell counts and differential white cell counts were made in the routine manner. The number of nucleated red corpuscles seen in differentially counting 200 white cells was recorded.

Table I presents a summary of the individual protocols of rabbits 2 to 13 inclusive. The total amount of silver-salvarsan injected varied from 469 to 2,300 milligrams containing a total amount of silver varying from 67.5 to 331.2 milligrams. The total amount of silver retained (found by Myers after

TABLE I

SILVER-SALVARSAN INTRAVENOUSLY—REPEATED ADMINISTRATION—66.7 MILLIGRAMS PER KILOGRAM

RABBITS 2-13

NO.	NUMBER OF INJECTIONS	TOTAL AMOUNT SILVER-SALVARSAN INJECTED	TOTAL AMOUNT SILVER INJECTED	TOTAL SILVER RETAINED		DURATION OF EXPERIMENT	INTERVAL AFTER LAST INJECTION	WEIGHT			RESULT
				AMOUNT	PER CENT			INITIAL	AT LAST INJECTION	FINAL	
		mg.	mg.	mg.		days	days	grams	grams	grams	
2	7	1030	149.3	38.8	25.98	44	8	2455	1765	1310	Killed
3	13	1554	225.33	21.0	9.59	52	10	2410	1670	1735	Killed
4	13	1236	179.2	8.4	4.70	62	7	2520	2290	2240	Killed
5	15	1594	231.13	7.5	3.26	55	7	2270	1755	1795	Killed
6	19	1661	240.85	19.1	7.93	78	7	1575	1600	1650	Killed
7	13	1520	225.4	24.1	10.70	51	7	1720	1940	2020	Killed
8	19	2300	331.2	43.5	13.10	76	12	1935	1885	1995	Killed
9	9	1169	168.3	5.9	3.50	36	6	1815	2030	1980	Killed
10	10	1415	203.8	43.4	21.30	37	5	2170	1900	1540	Died
11	5	469	67.54	12.3	18.20	19	4	1410	1460	1180	Killed
12	9	1217	175.3	14.8	8.44	32	3	2130	1800	1657	Died
13	11	1249	179.86	8.8	4.90	53	16	1755	1800	2120	Killed

incineration) varied from 5.9 to 43.5 milligrams and represented from 3.5 to 25.98 per cent of the total amount administered. Five of the animals gained in weight during the course of the injections and seven lost in weight. Two of the animals died of an intercurrent bronchopneumonia before the completion of the experiment. Nine were sacrificed with chloroform at the end of the experiment.

Rabbit 2 (March 19, 1921). Necropsy.—Animal is poorly nourished. Skin, mucous membranes and conjunctivae are intact and show no abnormal pigmentation. Subcu-

Fig. 1.—Liver from rabbit given nineteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram (20 dose) within 64 days. Silver granules are deposited in the Kupffer cells between the liver cell columns. They appear black in the photomicrograph. × 215 magnification.

taneous and omental fats are scanty though normal in color. Serous surfaces are normal. No increase in serous fluids. Blood clots normally. *Heart* normal in gross. Microscopically the endomysium contains a few isolated silver-holding phagocytes. *Lungs* contain many small areas of bronchopneumonia. No abnormal pigmentation in gross or in section. *Stomach* and *intestines* appear normal in gross. Microscopically the tunica propria (stomach and small intestine) contains many groups of silver granules and a few silver-holding phagocytes. The silver granules are situated almost entirely beneath the lining epithelium. *Liver* shows a definite increased pigmentation. The cut section is deep chocolate brown in color. Gall bladder and bile ducts are normal. Microscopically the liver cells around the efferent veins contain fat droplets, the periportal connective tissue is increased, the bile ducts are proliferated and there is an accumulation of small round cells. The Kupffer cells are richly loaded with dark brown silver granules. There are also many extracellular groups of silver granules between the liver cell columns and in the periportal connective tissue. A few of the parenchyma cells contain silver granules. *Spleen* appears normal in size. On cut section it is chocolate brown in color. Microscopically the pulp is rarefied and contains many free groups of silver granules and a few silver-holding phagocytes. Malpighian bodies are normal. *Pancreas* normal in gross. *Adrenals* normal in gross and in section. *Kidneys* are somewhat swollen. On cut section the capsule bulges, striations are regular although somewhat obscured. Microscopically the parenchyma cells are swollen and granular. Glomeruli appear normal. Interstitial tissue contains many fine silver granules. *Bone marrow* of femur is granular and uniformly reddish chocolate brown in color. Microscopically it is cellular, vascular and contains only a few fat cells. There are numerous phagocytes heavily loaded with silver granules and groups of silver granules lying free in the tissue. Brain and meninges appear normal in gross. Cerebrum normal in section.

Rabbit 3 (May 4, 1921). Necropsy.—Animal is poorly nourished. Skin, mucous membranes, conjunctivae and serous surfaces show no abnormal pigmentation. Heart and lungs are normal in gross and in section. There is an excess of peritoneal fluid. *Liver* is definitely cirrhotic and deep chocolate brown in color. Gall bladder and bile ducts appear normal. Microscopically the Kupffer cells contain an abundance of silver granules. There are also extracellular groups of silver granules between the liver cell columns and in the periportal connective tissue. *Spleen* shows increased pigmentation. The pulp contains many free groups of silver granules and a few silver-holding phagocytes. *Stomach* and *small intestine* normal except for the silver granules in the tunica propria. The groups of silver granules and silver-containing phagocytes are situated beneath the lining epithelium. *Pancreas* and *adrenals* are normal. *Kidneys* normal except for increased pigmentation. The silver granules are situated in the interstitial tissue. *Bone marrow* of femur is uniformly deep chocolate reddish brown in color. Microscopically it is very cellular and heavily sprinkled with silver phagocytes and extracellular groups of silver granules. Brain and meninges normal.

Rabbit 4 (May 26, 1921). Necropsy.—Animal is fairly well nourished. Serous cavities are normal. Heart and lungs normal in gross and in sections. Stomach and small intestine are normal. *Liver* and *spleen* normal except for slight increased pigmentation. Microscopically some of the Kupffer cells contain silver granules. The spleen pulp contains many free groups of silver granules and a few silver-holding phagocytes. Gall bladder and bile ducts normal. Pancreas and adrenals normal. Kidneys are normal except for slight increased pigmentation. The silver granules are contained in the interstitial tissue of both the cortex and medulla. *Bone marrow* of femur is deep chocolate reddish brown in color. Microscopically it contains many groups of silver granules.

Rabbit 5 (May 19, 1921). Necropsy.—Liver, spleen and kidneys show increased pigmentation. Kupffer cells, spleen pulp and interstitial tissue of kidneys contain silver granules. The kidneys contain a few scattered small areas radially arranged, which show marked interstitial lesions, accompanied by epithelial changes and accumulation of round cells. Bone marrow of femur uniformly deep chocolate brown. Microscopically the marrow is heavily sprinkled with groups of silver granules.

Rabbit 6 (June 13, 1921). Necropsy.—Skin, mucous membranes, conjunctivae show no abnormal pigmentation. Serous cavities normal. Heart and lungs normal in gross and in sections. Liver and kidneys show increased pigmentation. Gall bladder and bile ducts normal. Stomach and small intestine contain groups of silver granules in the tunica propria. Bone marrow of femur is hyperplastic and shows the usual silver granules. Necropsy is otherwise negative.

Rabbit 7 (May 27, 1921). Necropsy.—Negative except for increased pigmentation of the liver, kidneys, spleen and bone marrow accompanied microscopically with the deposition of silver granules. The Malpighian bodies in the spleen as well as the stroma con-

Fig. 2.—Bone marrow from rabbit given thirteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within a period of 42 days. Silver granules are deposited in extracellular groups and in many phagocytes. High dry lens magnification.

tain many groups of silver granules. The kidneys show a few radially arranged areas of cellular infiltration. The intervening glomeruli and parenchyma cells are normal.

Rabbit 8 (July 5, 1921). Necropsy.—Skin, mucous membranes and conjunctivae normal. Blood flows and clots normally. Serous cavities normal. Heart and lungs normal and show no silver microscopically. Liver, spleen and bone marrow are heavily pigmented. Microscopically the silver granules show the usual distribution. The tunica propria of the stomach and small intestine contains many groups of silver granules. Adrenals are normal in gross and in section. Brain and meninges are normal.

Rabbit 9 (June 6, 1921). Necropsy.—Negative except for the increased pigmentation
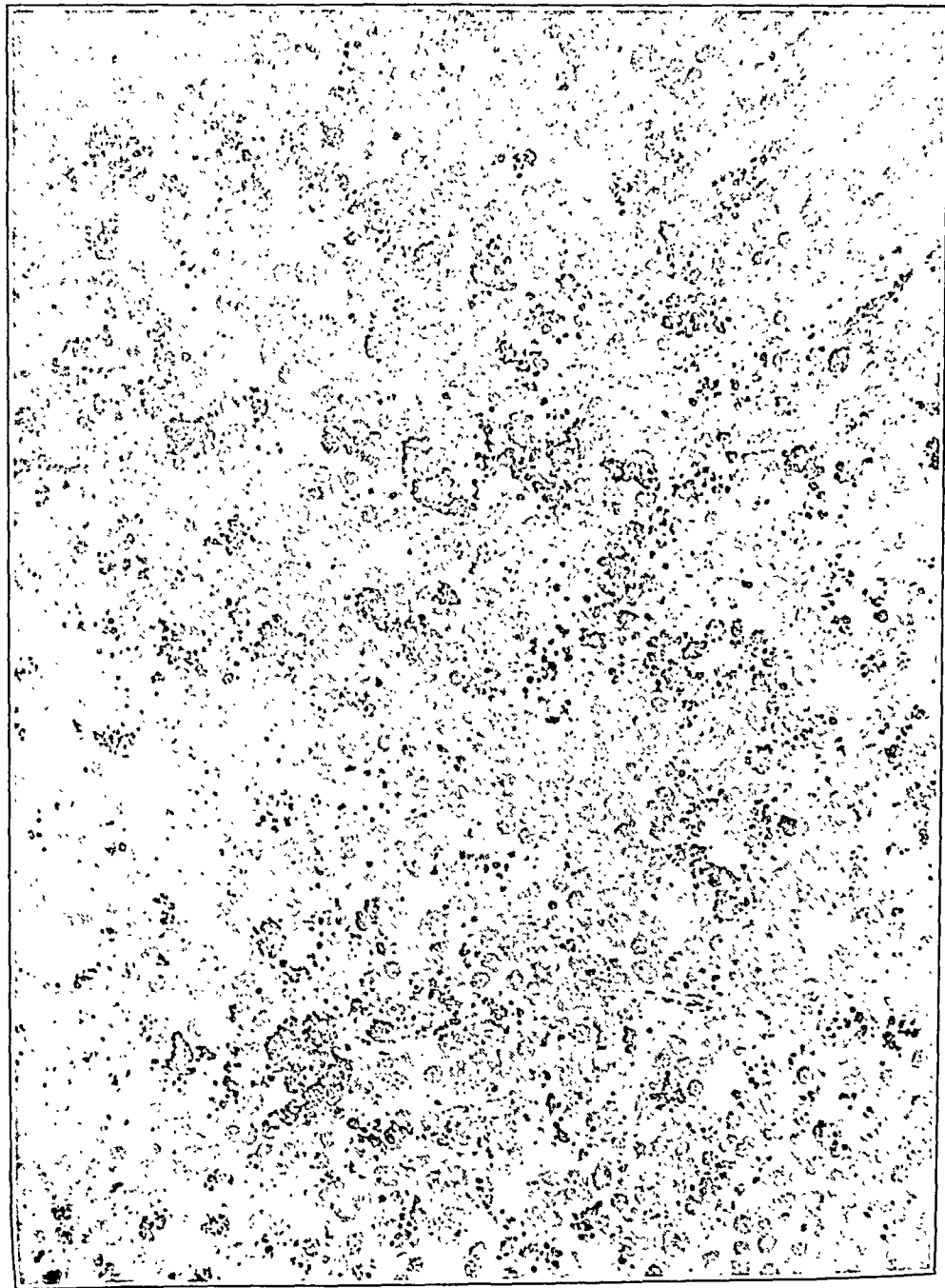
Fig. 3.—Spleen from rabbit given ten intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within a period of 32 days. The pulp contains many extracellular groups of silver granules and a few silver-holding phagocytes. High dry lens magnification.

of the liver, spleen, kidneys and bone marrow. Microscopically the deposition of silver granules is not as extensive as in Rabbit 8.

Rabbit 10 (June 14, 1921). Necropsy—Animal is poorly nourished. Considerable postmortem degeneration. Some excess of pleural fluid. *Lungs* show extensive broncho-pneumonia. The upper lobe of the left lung is completely consolidated. Heart is normal. Stomach and intestines appear normal. Microscopically the tunica propria of the stomach and small intestine contains many groups of silver granules and a few silver-holding phagocytes. *Spleen* on section is chocolate brown in color. Microscopically the pulp contains many extracellular groups of silver granules and a few silver-holding phagocytes.
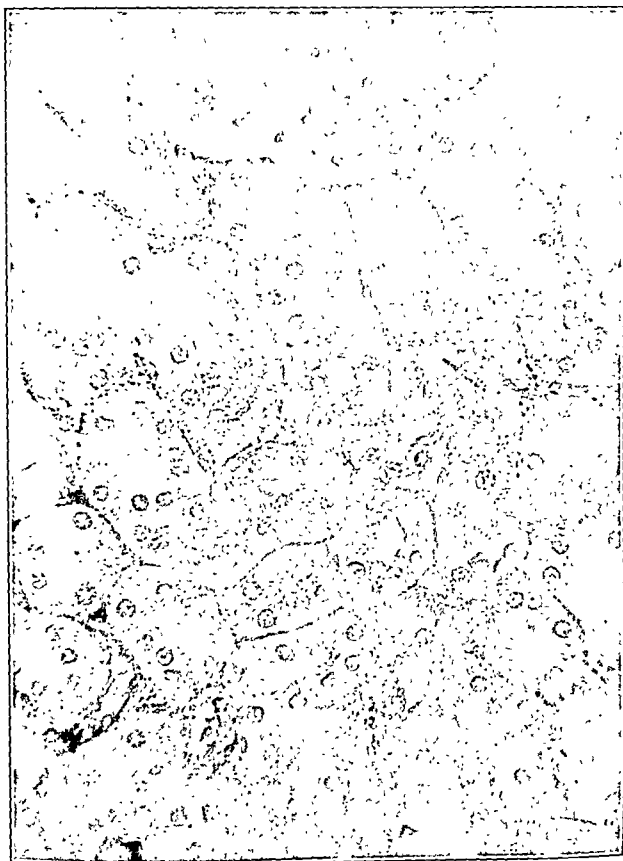
Fig. 4.—Kidney from a rabbit given thirteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within a period of 42 days. The silver granules are deposited in the interstitial tissue. High dry lens magnification.

Liver shows a markedly increased pigmentation. Gall bladder and bile ducts normal. Liver sections show a definite increase in periportal connective tissue with an accumulation of round cells. Some of the liver lobules are completely surrounded by a narrow band of fibrous tissue. The Kupffer cells are loaded with silver granules and there are many extracellular groups of silver granules between the liver cell columns and the interlobular connective tissue. *Kidneys* are swollen and show an increased pigmentation. The kidney sections show silver granules in the interstitial tissue. The tubular epithelium is swollen and granular and shows no necrosis. Some of the capsular spaces of the glomeruli

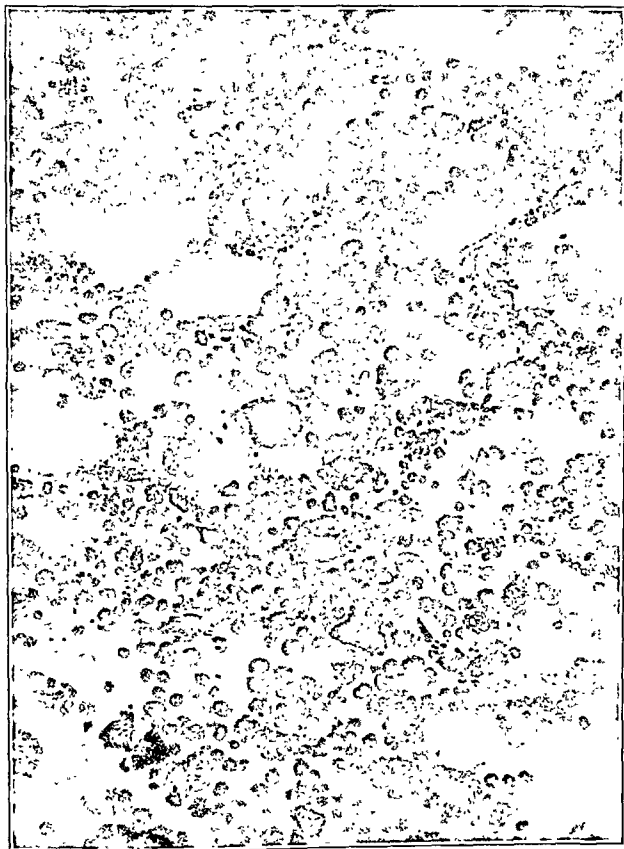Fig. 5.—Villi of small intestine from a rabbit given fifteen intravenous injections of 66.7 milligrams silver-salvarsan per kilogram within 45 days. The corium contains extracellular groups of silver granules and few silver-holding phagocytes. High dry lens magnification.

contain an albuminous precipitate. *Bone marrow* of femur is reddish chocolate brown throughout. Microscopically it is very cellular and contains a large amount of silver.

Rabbit 11 (May 27, 1921). Necropsy.—Animal is poorly nourished. No abnormal pigmentation of the skin or mucous membranes. Heart is normal. *Lungs* show a few small areas of bronchopneumonia. Stomach, intestines and adrenals are normal. *Liver* is cirrhotic. Gall bladder and bile ducts normal. Microscopically many of the Kupffer cells contain silver granules and also a few of the parenchyma cells. There are also a few granules in the increased periportal connective tissue. *Spleen* is normal in size and shows only a slight increased pigmentation. Microscopically the pulp contains a few free groups of silver granules and also a few pigment-holding phagocytes. *Kidneys* are somewhat swollen and show a slight increased pigmentation. Microscopically the parenchyma cells are swollen and granular. The glomeruli appear normal. There are a few silver granules in the basement membrane of the tubules and interstitial connective tissue. *Bone marrow* of femur is hyperplastic and shows considerable silver. Brain and meninges are normal. Cerebrum sections normal.

Rabbit 12 (June 19, 1921). Necropsy.—Animal is poorly nourished. Heart is normal, the right auricle is filled with firm blood clot. *Lungs* show extensive bronchopneumonia. Thymus is normal in gross and in sections. *Liver* is cirrhotic. Liver sections show the usual silver deposit. Spleen sections are normal except for the groups of silver granules scattered through the stroma. Adrenals are normal. *Kidneys* are somewhat swollen and show a slight increased pigmentation. Microscopically the kidney cells are swollen and granular and show no necrosis. The basement membrane of the tubules and interstitial tissue contains silver granules. *Bone marrow* is quite cellular and heavily sprinkled with small groups of silver granules. Brain and meninges are normal.

Rabbit 13 (July 27, 1921). Necropsy.—Negative except for the increased pigmentation of the liver, spleen, bone marrow and kidneys. Kidney sections show several areas of cellular infiltration. Bone marrow of femur is uniformly hyperplastic and heavily pigmented.

Rabbit 14. Necropsy.—Skin, mucous membranes and conjunctivae normal. Serous surfaces normal. Heart is normal. Heart sections show no silver. Lungs are negative except for a few silver granules in the interlobular connective tissue. *Stomach* and *intestines* are normal in the gross. Microscopically there are a few groups of silver granules in the tunica propria of the stomach and small intestine. Spleen sections show a few groups of silver granules in the pulp. Liver shows an increase in periportal connective tissue and many of the Kupffer cells contain silver granules. Kidneys are normal. Microscopically the connective tissue stroma contains a few silver granules. Bone marrow of femur is cellular and contains several groups of silver granules. Brain and meninges are normal.

Rabbit 15. Necropsy.—Negative except for slight increased pigmentation of the liver, spleen, kidneys and bone marrow.

Rabbit 16. Necropsy.—Skin, mucous membranes, conjunctivae and serous surfaces normal. Thymus, heart and lungs normal. Stomach and duodenum sections show a few groups of silver granules in the fibroreticular stroma of the corium. *Liver* is cirrhotic. Kupffer cells and periportal connective tissue contain silver granules. Spleen pulp contains a few groups of silver granules. Adrenals are normal. *Kidneys* show scattered, radially arranged interstitial lesions with the formation of small depressions on the outer surface. These areas also show epithelial alterations, with infiltration of round cells. In the non-affected areas the parenchyma cells and glomeruli are normal. Bone marrow of femur appears somewhat hyperplastic and contains many groups of silver granules. Brain and meninges normal. Cerebrum sections show no change.

Rabbit 17. Necropsy.—No external abnormal pigmentation. Serous cavities are normal. Heart and lungs normal. Stomach, small intestines, liver, spleen and kidneys contain deposits of silver granules. Bone marrow is of the mixed type and contains many groups of silver granules. Necropsy otherwise negative.

TABLE II

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 2531 GRAMS. TOTAL AMOUNT OF SILVER GIVEN = 362.4 MG. PER CENT OF SILVER RETAINED = 2 (MYERS)

RABBIT 14

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				
							LYMPHO-CYTES	LARGE MONOS	NEUTRO-PHILES	BASO-PHILES	NUCLEATED RED CELLS
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	115	1730	70	5,080,000	0.70	7,000	37.5	5.5	55.5	1.5	0
4	113	1690									
7	114	1705	65	4,408,000	0.74	12,000	57.0	6.0	34.5	2.5	0
10	120	1795									
14	125	1850	67	4,544,000	0.74	11,800	55.0	6.0	35.0	4.0	1
17	126	1840									
21	127	1900	80	4,848,000	0.83	11,950	48.5	7.5	38.5	5.5	0
24	127	1910									
28	124	1860	85	5,928,000	0.72	14,950	59.0	5.5	30.0	5.5	0
31	127	1905									
35	134	2015	94	5,664,000	0.84	9,600	39.0	8.0	48.5	4.5	0
38	134	2010									
42	141	2120	103	6,520,000	0.79	12,250	47.0	6.5	37.0	9.5	0
45	142	2125									
49	149	2230	98	6,224,000	0.79	10,900	42.0	6.0	49.5	2.5	0
52	149	2230									
56	152	2275	97	6,848,000	0.71	9,200	43.0	4.5	46.5	6.0	0
59	153	2290									
63	162	2430	87	5,464,000	0.81	7,350	46.5	5.0	45.5	3.0	1
69		2400	84	5,568,000	0.76	9,900	62.0	7.5	29.5	1.0	2
77		2410	89	5,528,000	0.81	8,450	51.0	5.5	38.0	5.5	0
83		2400	91	5,936,000	0.77	8,650	48.5	6.0	41.5	4.0	0
91		2475	95	5,720,000	0.83	14,150	51.5	5.5	40.5	2.5	0
98		2455	88	5,328,000	0.83	9,650	55.5	7.5	30.5	6.5	0
101		2380	92	5,488,000	0.85	10,800	43.5	4.5	48.5	3.5	0
105		2440	88	5,344,000	0.83	11,600	54.5	3.0	40.0	2.5	0

105th day (October 25, 1921) Active and apparently normal. Sacrificed with chloroform.

TABLE III

66.7 mg. Silver-Salvarsan per Kilogram Intravenously, One Per Cent Water Solution. Total Amount Silver-Salvarsan Given = 2.369 Grams. Total Amount of Silver Given = 238.8 mg. Per Cent or Silver Retained = 1.52 (Myers)

RABBIT 15.

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	COLOR INDEX	RED CELLS PER C.MM.	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHO-CYTES	LARGE MONOS	NEUTRO-PHILES	BASO-PHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	127	1905	92	0.71	6,572,000	13,900	39.5	6.5	50.5	3.5	0
4	135	2030	97	0.70	6,904,000	12,450	45.5	5.0	48.0	1.5	0
7	137	2060	96	0.72	6,760,000	10,200	59.0	13.5	23.5	4.0	0
10	136	2040	94	0.67	7,056,000	9,450	46.5	7.5	43.5	2.5	0
14	138	2070	96	0.66	7,360,000	7,800	56.5	4.5	32.5	6.5	0
17	138	2070	96	0.70	6,936,000	12,050	37.0	8.0	46.0	9.0	0
21	144	2140	97	0.72	6,752,000	7,800	56.5	4.0	36.5	3.0	0
24	154	2315	90	0.78	5,800,000	6,800	57.5	1.5	34.0	7.0	1
28	149	2235	85	0.80	5,336,000	10,000	54.5	7.0	34.0	4.5	0
31	149	2240	88	0.81	5,480,000	9,100	50.5	7.0	31.5	11.0	0
35	146	2200	84	0.84	5,072,000	11,150	53.5	5.5	35.0	6.0	0
38	163	2440	89	0.80	5,600,000	8,900	69.0	5.5	22.5	3.0	0
42	162	2410	95	0.86	5,504,000	9,000	58.0	6.5	34.5	1.0	0
45	160	2400	100	0.79	6,360,000	11,500	68.0	6.0	22.0	4.0	0
49	163	2480									
52	166	2490									
56		2525									
63		2635									
69		2640									
77		2785									
83		2790									
91		2800									

91st day (October 14, 1921) Active and apparently normal.   Killed with chloroform.

TABLE IV

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION, TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 2.363 GRAMS. TOTAL AMOUNT SILVER GIVEN = 337.9 MG. PER CENT OF SILVER RETAINED = 1.2 (MYERS)

RABBIT 16

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN mg.	BODY WEIGHT grams	HB. per cent	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				
							LYMPHO-CYTES per cent	LARGE MONOS per cent	NEUTRO-PHILES per cent	BASO-PHILES per cent	NUCLEATED RED CELLS
1	116	1745	86	7,592,000	0.57	14,300	53.0	8.0	37.0	2.0	0
4	119	1765									
7	118	1765	86	7,248,000	0.60	10,110	40.5	6.5	50.5	2.5	0
10	114	1715									
14	118	1770	79	6,528,000	0.61	10,800	45.5	6.5	46.0	2.0	0
17	117	1750									
21	116	1740	80	6,304,000	0.63	15,200	43.5	8.0	43.0	5.5	0
24	117	1750									
28	116	1745	79	6,048,000	0.65	22,000	51.0	5.0	40.5	3.5	2
31	116	1750									
35	115	1720	83	6,032,000	0.69	12,400	46.0	8.0	39.0	7.0	4
38	117	1755									
42	119	1770	88	7,000,000	0.63	13,600	44.5	4.0	48.5	3.0	1
45	120	1795									
49	120	1790	83	6,520,000	0.64	18,850	39.0	4.0	55.5	1.5	4
52	123	1790									
56	117	1750	81	6,136,000	0.66	16,650	49.0	2.5	45.5	3.0	0
59	120	1800									
63	122	1835	84	6,856,000	0.62	15,900	45.0	12.5	40.0	2.5	0
66	123	1845									
70		1875	80	6,512,000	0.62	14,950	36.0	8.0	53.5	2.5	1
78		1866	95	7,212,000	0.65	12,100	40.0	6.0	50.0	4.0	0
84			100	7,808,000	0.64	11,100	38.0	6.5	47.5	8.0	0
91		1866	103	8,000,000	0.64	15,300	44.5	5.0	47.5	3.0	0
101		1870	94	7,128,000	0.66	12,200	45.5	6.5	44.5	3.5	0
105		1920	91	6,896,000	0.67	11,600	55.5	3.5	39.0	2.0	0
108		1950	95	6,840,000	0.69	13,000	43.0	2.5	53.0	1.5	0

108th day (October 28, 1921). Good condition and active. Sacrificed with chloroform.

TABLE V

66.7 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 1.12 GRAMS. TOTAL AMOUNT OF SILVER GIVEN = 160.2 MG. PER CENT SILVER RETAINED = 2.5 (MYERS)

RABBIT 17

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				
							LYMPHO-CYTES	LARGE MONOS	NEUTRO-PHILES	BASO-PHILES	NUCLEATED RED CELLS
	mg.	grams	per cent				per cent	per cent	per cent	per cent	per cent
1	113	1700	84	7,000,000	0.60	8,100	43.0	8.5	45.5	3.0	0
4	115	1730	89	7,240,000	0.61	8,900	48.0	5.0	45.0	2.0	0
7	120	1795	81	6,068,000	0.68	7,250	45.5	16.5	33.5	4.5	0
10	121	1800	76	5,604,000	0.68	12,100	50.5	6.5	41.0	2.0	2
14	117	1820	76	6,096,000	0.63	9,950	37.0	5.5	55.5	2.0	0
17	108	1730									
21	109	1620									
24	100	1620									
28	97	1495									
31		1455									
35		1340	80	6,112,000	0.66	10,200	46.5	6.5	45.0	2.0	0
42		1270	86	7,128,000	0.61	12,000	32.5	1.5	64.0	2.0	0
49		1490	75	6,304,000	0.60	9,300	59.5	2.0	32.5	6.0	0
56		1630	79	6,272,000	0.64	10,830	50.0	4.5	43.5	6.0	0
63		1740	87	7,072,000	0.62	15,800	58.0	3.5	35.5	3.0	0
69		1860	83	6,456,000	0.65	9,600	57.0	4.0	36.5	2.5	0
73		1870	80	6,080,000	0.67	6,500	43.5	3.0	52.0	1.5	0

73rd day (September 23, 1921) Active. Killed with chloroform.

TABLE VI

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, TWO PER CENT SOLUTION IN 0.4 PER CENT SODIUM CHLORIDE. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 0.276 GRAMS. TOTAL AMOUNT SILVER GIVEN = 39.5 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS).

RABBIT 18

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				
							LYMPHO-CYTES	LARGE MONOS	NEUTRO-PHILES	BASO-PHILES	NUCLEATED RED CELLS
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	21.0	2100	96	7,608,000	0.63	9,150	61.5	3.5	31.5	3.5	0
5	20.8	2080									
8	21.8	2180	100	7,784,000	0.65	8,600	69.0	4.0	24.5	2.5	0
12	21.6	2170									
15	21.6	2165	101	7,088,000	0.72	8,550	67.5	4.0	27.0	1.5	0
19	22.8	2280									
22	22.8	2290	98	6,456,000	0.77	9,450	63.0	2.5	30.0	4.5	0
26	23.6	2365									
29	24.0	2410	99	6,064,000	0.83	7,650	71.5	2.5	24.0	2.0	0
33	25.0	2465									
36	25.0	2485	102	6,584,000	0.78	9,550	64.5	2.0	30.0	3.5	0
40	26.0	2640									
43		2660	99	6,144,000	0.81	7,200	62.0	1.5	32.0	4.5	0
50		2740	104	6,672,000	0.79	11,050	68.0	4.5	22.5	5.0	0
57		2830	107	7,952,000	0.68	13,350	55.5	3.0	34.5	7.0	0
64		2890	105	7,672,000	0.69	13,450	69.5	3.0	25.0	2.5	0
71		2940	103	6,744,000	0.77	9,450	62.5	3.5	29.0	2.5	0
78		2960	104	6,448,000	0.81	12,200	64.5	7.0	24.0	5.0	0
82		2930	104	6,208,000	0.84	10,550	47.0	2.5	45.0	4.5	0
84		3010	102	6,552,000	0.78	7,650	63.5	2.0	31.5	5.5	0

84th day (November 3, 1921) Excellent condition. Sacrificed with chloroform.

TABLE VII

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, TWO PER CENT WATER SOLUTION. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 0.223 GRAM. TOTAL AMOUNT OF SILVER GIVEN = 31.0 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS)

RABBIT 19

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				
							LYMPHO-CYTES	LARGE MONOS	NEUTRO-PHILES	BASO-PHILES	NUCLEATED RED CELLS
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	16.4	1635	85	5,944,000	0.72	9,050	53.5	5.0	37.5	4.0	0
5	17.4	1735									
8	17.0	1695	85	5,908,000	0.72	10,000	67.0	2.5	26.5	4.0	0
12	16.9	1820									
15	18.0	1800	93	6,200,000	0.75	14,050	60.5	2.5	34.0	3.0	0
19	18.4	1840									
22	18.4	1830	85	5,552,000	0.77	11,100	62.0	1.5	33.0	3.5	0
24	19.0	1900									
29	19.0	1895	88	6,072,000	0.73	15,050	36.0	4.5	33.5	4.0	0
33	20.0	1995									
36	19.6	1965	96	6,248,000	0.77	13,330	60.0	10.0	24.5	5.5	0
40	21.0	2090									
43		2115	95	6,440,000	0.74	10,850	61.5	2.0	33.5	3.0	0
47		2200	98	6,216,000	0.79	11,900	67.0	5.0	23.0	5.0	0

47th day (September 27, 1921). Active and normal. Killed with chloroform.

TABLE VIII

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, TWO PER CENT SOLUTION IN 0.4 PER CENT SODIUM CHLORIDE. TOTAL AMOUNT SILVER-SALVARSAN GIVEN = 0.297 GRAMS. TOTAL AMOUNT SILVER GIVEN = 42.5 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS)

RABBIT 20

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	RED CELLS PER C.MM.	COLOR INDEX	WHITE CELLS PER C.MM.	LYMPHOCYTES	LARGE MONOS	NEUTROPHILES	BASOPHILES	NUCLEATED RED CELLS
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	22.1	2210	74	5,208,000	0.71	8,550	57.5	10.0	31.0	1.5	0
5	23.0	2390									
8	22.5	2245	73	4,608,000	0.79	8,050	61.5	7.5	28.5	2.5	0
12	24.0	2405									
15	24.0	2365	81	5,376,000	0.76	10,950	51.0	8.0	40.0	1.0	0
19	26.0	2585									
22	25.0	2515	81	5,052,000	0.81	7,150	50.5	3.5	43.0	3.0	0
26	24.0	2375									
29	26.0	2585	82	5,112,000	0.80	16,850	47.0	3.5	45.5	4.0	0
33	26.0	2680									
36	27.0	2680	82	5,312,000	0.77	11,950	42.0	6.0	50.5	1.5	0
40	27.0	2700									
43		2760	82	5,032,000	0.82	7,700	57.0	6.5	35.0	1.5	0
50		2780	93	5,824,000	0.80	12,050	61.5	4.5	33.0	1.0	0
57		2875	97	6,168,000	0.80	11,900	56.5	5.0	34.0	4.5	0
64		3010	96	5,704,000	0.84	10,350	50.5	9.0	39.0	1.5	0
71		2840	98	6,176,000	0.80	10,200	35.5	6.5	56.5	1.5	0
78		2960	92	5,544,000	0.84	11,400	61.5	4.0	32.5	2.0	0
82		2885	95	5,784,000	0.83	8,400	53.0	1.5	41.5	4.0	0
84		2940	98	6,168,000	0.80	10,100	54.0	5.5	38.0	2.5	0

84th day (November 3, 1921) Excellent condition. Sacrificed with chloroform.

TABLE IX

10 MG. SILVER-SALVARSAN PER KILOGRAM INTRAVENOUSLY, ONE PER CENT WATER SOLUTION. TOTAL AMOUNT OF SILVER-SALVARSAN GIVEN = 0.234 GRAM. TOTAL AMOUNT OF SILVER GIVEN = 33.4 MG. AMOUNT OF SILVER RETAINED = TRACE (MYERS)

RABBIT 21

DAY OF EXPERIMENT	SILVER-SALVARSAN GIVEN	BODY WEIGHT	HB.	COLOR INDEX	RED CELLS PER C.MM.	WHITE CELLS PER C.MM.	DIFFERENTIAL COUNT				NUCLEATED RED CELLS
							LYMPHO-CYTES	LARGE MONOS	NEUTRO-PHILES	BASO-PHILES	
	mg.	grams	per cent				per cent	per cent	per cent	per cent	
1	17.4	1733	77	0.61	6,384,000	6,750	63.5	6.5	27.0	3.0	0
5	17.0	1690									
8	17.4	1780	73	0.73	5,752,000	4,450	51.0	4.0	30.5	8.5	0
12	18.6	1865									
15	19.2	1920	88	0.72	6,176,000	7,200	64.0	3.5	30.5	2.0	1
19	19.4	1930									
22	19.8	1970	82	0.72	5,744,000	6,500	68.5	6.5	21.0	4.0	0
26	19.6	1965									
29	20.6	2060	87	0.74	5,992,000	15,200	50.5	5.5	38.0	6.0	0
33	21.0	2100									
36	21.0	2105	91	0.72	6,336,000	8,050	54.5	6.5	32.5	6.5	0
40	22.8	2285									
43		2320	87	0.73	6,072,000	8,950	67.5	3.5	25.0	4.0	0
50		2500	90	0.69	6,504,000	12,750	68.0	5.5	25.0	1.5	0
57		2475	90	0.66	6,864,000	12,100	66.5	5.5	24.5	3.5	0
64		2525	96	0.72	6,744,000	9,250	56.0	7.0	31.0	6.0	0
71		2560	98	0.79	6,296,000	11,600	59.5	3.0	34.5	3.0	0
78		2660	94	0.78	6,058,000	10,300	63.0	3.5	32.5	1.0	0
83		2680	94	0.70	6,752,000	8,550	69.5	2.0	25.0	3.5	0
84		2735	100	0.72	6,968,000	9,800	51.0	8.5	38.0	2.5	0

84th day (November 3, 1921) Excellent condition. Killed with chloroform.

Rabbit 18. Necropsy.—Entirely negative except for a mild degree of liver cirrhosis. A few of the Kupffer cells contain silver granules.

Rabbit 19. Necropsy.—Liver and spleen contain a few silver granules. Kidneys show scattered areas of round cell infiltration. The intervening kidney parenchyma is normal. Bone marrow of femur contains a few groups of silver granules. Necropsy otherwise negative.

Rabbit 20. Necropsy.—Negative except that the liver, spleen and bone marrow contain a very few silver granules. Kidneys show a few scattered scars radially arranged in which there are marked interstitial and parenchymatous alterations. The intervening kidney tissue is normal.

Rabbit 21. Necropsy.—Entirely negative.

DISCUSSION

The experiments yielded no evidence that the repeated intravenous administration of silver-salvarsan into rabbits in doses of 10 milligrams per kilogram (equivalent to a clinical dose of 0.6 gram) or 66.7 milligrams per kilogram (equivalent to a clinical dose of 4 grams) produces any severe tissue alterations.

All of the rabbits that remained healthy and gained in weight during the course of the injections presented no pathologic tissue alterations at necropsy which could be attributed to silver-salvarsan with the exception of the deposit of silver granules found mainly in the liver, spleen, bone marrow, and kidneys. The amount of silver found on histologic examination corresponded very closely with the amounts found chemically after incineration by Myers. The animals that lost in weight showed kidney and liver lesions at necropsy and as a result a much greater percentage of the silver was retained in the tissues.

Before drawing any histopathologic deductions in regard to the tissue changes that may be attributed to the silver-salvarsan, one should first eliminate the pathologic lesions that occur with a certain regularity in the outwardly normal rabbit. In view of the importance of the occurrence of spontaneous lesions in the kidneys and in the livers of rabbits, Doctor Wm. Ophüls, 1910-11, Professor of Pathology, Leland Stanford Junior University School of Medicine, made a careful study of these organs and incidentally of the heart and of the aorta in fifty rabbits. He states that the animals used were partly fresh animals from the market and partly animals raised in the laboratory. He further states that "Several old rabbits that had been in the laboratory for a year or longer were especially selected on account of the greater likelihood of the existence of renal or hepatic lesions in them. Twenty-eight rabbits, among them some of the old ones had entirely normal kidneys, nine showed parenchymatous lesions, three a few areas of cellular infiltration. In ten we found scattered small areas in which were marked interstitial lesions with the formation of small depressions on the surface. Four of these proved to be radially arranged chronic septic foci which extended from the vicinity of the papilla to the outer surface. In the other six cases the same arrangement of the newly formed connective tissue in narrow radial bands which started deep in the pyramids was noticeable although no evidence of

a septic infection could be discovered. The interstitial lesions were accompanied by marked epithelial lesions in two cases only, producing to a certain extent the picture of a chronic parenchymatous nephritis, although the lesions were never very extensive. Lesions of the blood vessels or primary lesions of the glomeruli were not found in any case. Only six normal livers were encountered; the rest showed various stages of coccidial infection with more or less cirrhosis and sometimes cirrhotic processes in which coccidia could not be demonstrated. We are very strongly of the opinion that our findings throw very serious doubt on the numerous reports of the experimental production of cirrhosis in the liver of rabbits as this animal would seem to be entirely unsuitable for such experiments. No gross lesions of the heart or of the aorta were discovered in any of the animals.''

Our experience in the routine examinations of apparently normal control rabbits confirms that of Doctor Ophüls. We have also found that a large percentage of the rabbits have spontaneous kidney lesions and that more or less cirrhosis of the liver with round-cell infiltration is the rule rather than the exception. We have further encountered areas of round-cell infiltration in the heart muscle and brain, areas of bronchopneumonia and arteriosclerosis. It is evident therefore that all histopathologic deductions on rabbits should be made with a full knowledge of the occurrence of the spontaneous lesions. This would especially apply to experiments with the arsenic and mercury or bismuth compounds where the drugs are known to produce kidney or liver damage when administered in toxic doses.

The experiments described in this article were undertaken primarily to study the retention and elimination of silver following the repeated intravenous administration of silver-salvarsan The histopathologic examinations made at necropsy were undertaken with a full knowledge of the spontaneous lesions that frequently occur in the rabbit. The direct object of the histopathologic study was to determine whether silver-salvarsan in the doses administered produced any diffuse kidney lesions or any destructive lesions in the parenchyma cells of the liver and also to determine the sites of distribution of the silver granules. There were no diffuse kidney lesions encountered. The parenchyma cells of the liver were in all cases well preserved.

Our findings are essentially in agreement with those of Kolle, 1918, who states that Professor B. Fischer (Frankfurt a / M.) has determined that rabbits which had received more than 60 injections of 0.07 gram of silver-salvarsan per kilogram intravenously every second day showed no changes of the organs and Professor Goldstein observed also no changes in the nervous system. Symptoms of hypersensitiveness were also not observed in rabbits after injections of silver-salvarsan every second day.

Schlossberger, 1920, states, "In rabbits that received 0.02-0.04 gram silver-salvarsan per kilogram every 3-4 days for 9-12 months, with a total of 3-4 grams, no argyria of the skin could be demonstrated. The silver was deposited in the otherwise normal internal organs, especially in the Kupffer cells of the liver as shown by B. Fischer and to a slight extent also in the

kidneys. No functional injury could be demonstrated for the rabbits, which constantly increased in weight.''

In all of our rabbits that received repeated intravenous injections of 66.7 milligrams silver-salvarsan per kilogram the silver granules were deposited in the Kupffer cells of the liver, rarely in the liver cells proper, and frequently in extracellular groups between the liver cell columns and in the periportal connective tissue. The bone marrow tissue in proportion to its size contained even more silver than the liver. The granules were deposited in groups lying free in the tissue and in heavily loaded phagocytes. The spleen pulp contained many free groups of silver granules and a few silver-holding phagocytes. The interstitial tissue of the kidney contained many free silver granules and the tunica propria of the stomach and small intestine contained groups of silver granules and a few silver-holding phagocytes.

In the rabbits that received repeated intravenous injections of 10 milligrams silver-salvarsan per kilogram the silver deposits were inconspicuous. The silver granules were deposited in the sites enumerated above.

The blood examinations were carried out as a preliminary study to determine the effect silver-salvarsan exerts upon the cell elements of the blood. The results are given in detail in Tables II-IX. Blood volume determinations were not made on account of the unsuitability of the rabbit. Anemia was not produced in any of the eight experiments. The majority of the animals showed a gradual increase in hemoglobin and red cells. The white cell counts and differential counts remained within the normal limits of variation.

SUMMARY

Repeated intravenous administration of silver-salvarsan into rabbits in doses of 10 milligrams or 66.7 milligrams every 3-7 days for a period of 47-70 days did not produce any noteworthy tissue or blood alterations.

In the rabbits that received repeated intravenous injections of 66.7 milligrams silver-salvarsan per kilogram (20 times the average clinical dose) many silver granules were deposited in the Kupffer cells of the liver, in extracellular groups between the liver cell columns and in the periportal connective tissue. They were also found in the bone marrow, spleen pulp, interstitial tissue of the kidney and in the tunica propria of the stomach and small intestine.

In a relative way the amount of silver found on histologic examination of the tissues corresponded with the amount found chemically after incineration.

The great individual variation in the amount of silver found at necropsy may have been due to preexisting lesions in the liver and kidneys.

In the rabbits that received 10 milligrams silver-salvarsan per kilogram (3 times the average clinical dose) the silver deposits were inconspicuous.

REFERENCES

Kolle, W.: Experimentelle Studien zu Ehrlichs Salvarsantherapie der Spirochaetenkrank-
heiten und ueber neue Salvarsanpraeparate. Deutsch. med. Wchnschr., Nos. 43 &
44, p. 1177-1181, 1211-1215.
Myers, C. N.: 1923. Argyria and its Relation to Silver Therapy I. Argyria-Historical,
Am. Jour. Syph., vii, 125-144.
Myers, C. N., and Corbitt, H. B.: 1924. Retention and Elimination of Silver with Special
Reference to Silver-Salvarsan and Silver Therapy II. Am. Jour. Syph., viii, No. 3.
Ophüls, W.: 1910-11. Occurrence of Spontaneous Lesions in Kidneys and Livers of Rab-
bits and Guinea Pigs, Proc. Soc. Exper. Biol. and Med., viii, 75-77.
Schlossberger, H.: 1920. Angebliche, auf Kurpfuscherdiagnose gestuetzte, und nicht kon-
trollierte Argyrie nach Silber-Salvarsan. Therap. Halbmonat., xxxiv, 608-609.
Van Slyke, D. D.: 1918. Gasometric Determination of the Oxygen and Hemoglobin of
Blood, Jour. Biol. Chem., xxxiii, 127-132.

THE EFFECT OF HIGH CONCENTRATIONS OF ALKALI UPON THE COLOR OF PHENOLSULPHONEPHTHALEIN*

By Roger S. Hubbard, Ph.D., and D. Olan Meeker, B.S.,
Clifton Springs, N. Y.

IN the determination of the rate of phenolsulphonephthalein excretion a
method is used in this laboratory very similar to that originally described
by Rowntree and Geraghty[1]; a standard stock solution is prepared, and a
fresh portion of the standard is used in every test by comparing the color
produced by the dye in the urine with that of the standard in a colorimeter
of the plunger type (Leitz-Dubosq). In preparing the standard solution
1 c.c. of a sterile concentrated solution containing 6 mg. of the mono-sodium
salt of phenolsulphonephthalein† is diluted to 2 liters in the presence of
an excess of sodium hydroxide. Such a solution is ordinarily used up in
about 6 months.

It has been the custom to check the value of each new standard against
that of the one previously in use. In almost every instance the results cor-
responded perfectly; this can be illustrated by the following figures, which
are typical of those usually obtained. A solution containing an amount of
alkali only slightly in excess of that needed to develop the full color of the
dye was prepared on January 8, and this solution had not changed its
color, as determined by comparison with a new standard, on June 10. An-
other solution was prepared on March 3 in which an excess of 10 c.c. of
N/10 alkali over that needed to give the full color was used. Since the
final volume was 2 liters, this gave an excess concentration of alkali of
N/2000. This solution was also checked up on June 10 and showed no change
in color. These results are in accord with those described by Rowntree and
Geraghty.[1]

On one occasion, however, it was thought that the color of a standard solution had faded. A new solution was prepared and it was found that the old solution had lost about half its color in approximately two weeks. Inquiry brought out the fact that a much greater excess of alkali than usual had been used in preparing the poor standard. In order to avoid a repetition of such an error a standard solution containing the least possible excess of alkali over that needed to produce maximum color was prepared. This solution checked satisfactorily against other solutions at the time it was made, and rechecked satisfactorily after it had been in use about two months. Later the color appeared to decrease; the solution was then rechecked, and it was found that it had lost about 25 per cent of its intensity. A further excess of alkali was added to the solution, and the full intensity of the color was restored. It seemed as though the alkali had become neutralized in some way, possibly by exposure to carbon dioxide during the routine use of the solution. These experiences seemed to make it advisable to work out the effect of an excess of alkali upon the rate of fading of the color of phenol-sulphonephthalein. It was only when precautions were taken to insure the presence of the least possible excess of sodium hydroxide that fading took place in the presence of low concentrations of alkali, and even then the color did not change until after the solution had been in use for a fairly long time. It seemed to the authors that the addition of too much alkali was more likely to occur and our investigations have been confined to the effect of such additions.

A search of the literature was made for a description of findings similar to those encountered. In a paper by Brightman, Hopfield, Meacham, and Acree[2] published in 1918 a study was made of the endpoints and fading of phenolsulphonephthalein endpoints. They summed up the work of earlier investigators as follows on page 1,940: " * * * phenolsulphonephthalein and other indicators of this series * * * show very little, if any, fading in an *excess* of alkali." The italics occur in the original article. In the experimental part of the work they reported experiments in which 5 to 7 times as much alkali as was necessary to produce a complete change of the compound into its red-purple form were added, and showed by spectrophotometric methods that no fading took place in several days.

It seems probable that the sentence quoted above is intended to apply only to the effect of the presence of a small excess of alkali, for in a paper by Lubs and Acree[3] which was published in 1916 and a reference to which was given in the paper by Brightman et al just cited the following statement occurs on page 2,778. "It (the solution of phenolsulphonephthalein) is yellow in slightly acid solution, pink in stronger acids, and purple in weak alkalies. Stronger alkaline solutions discharge the color, probably through hydration, and the colorless solution becomes colored again on the addition of acids."

In the article by Rowntree and Geraghty[1] in which the use of the dye for the determination of renal function was first described, the following directions for preparing the standard are given on page 598. "The standard

solution used for comparison consists of 3 mg. of phenolsulphonephthalein (or ½ c.c. of the solution used for injection) diluted up to 1 liter and made alkaline by the addition of *only* one or two drops of 25 per cent NaOH solution. This is a beautifully purplish-red solution retaining its intensity for weeks or for an indefinite period." The parenthesis occurs in the original sentence, and the italics have been introduced by the writers of the present article. No more definite descriptions or discussion of the effect of strong alkali upon the dye than these were found.

For studying the effect of alkali upon the fading of the color of phenol-sulphonephthalein a concentrated solution of the dye was prepared by diluting 1 c.c. of the stock dye solution (6 mg. of the mono-sodium salt) to 250 c.c. with distilled water. Solutions for the experiments were prepared from this by adding sodium hydroxide to 25 c.c. portions until the maximum color was
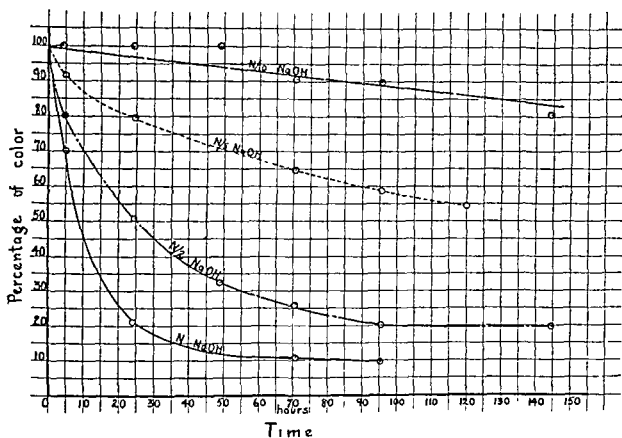

Chart I.

obtained (approximately 11 c.c. of N/100 sodium hydroxide were necessary) and diluting to 200 c.c. in the presence of enough sodium hydroxide to give a final excess concentration of normal, half normal, fifth normal and tenth normal alkali. Each of the solutions thus prepared contained a concentration of the dye equal to that in the standard used in the renal function test. The color of these alkaline solutions was compared from time to time with such standards as had been found satisfactory in the routine work, and with standards freshly prepared from the concentrated stock solution. The results are shown in the chart. The curves show that all of the solutions lost color on standing, and that the rate of the color change was proportional to the excess concentration of alkali present. The relationship between the curves suggests the conclusion that some definite chemical reaction has taken place. Lubs and Acree,[3] as cited above, have suggested that such changes are due

to hydration, and there is nothing in the results reported to refute such a suggestion. We confirmed the statement of these authors that the color is partially restored by the addition of acid to the faded solution, but found that the full color could not be restored in a short period of time by this method.

The experiments reported show that alkali when present in a concentration of N/10 or more destroys the color of phenolsulphonephthalein. The results discussed in the first part of the paper show that sufficient alkali must be used in preparing the solution if changes in its color are to be prevented. From the work as a whole it seems that a final normality of N/1000 to N/2000 alkali is best for maintaining a permanent standard, but it is probably wise to check the strength of the color of the dye from time to time to be sure that no deterioration has taken place. A buffered solution of suitable hydrogen-ion concentration and buffer content might be used advantageously, but is not necessary.

BIBLIOGRAPHY

[1] Rowntree, L. G., and Geraghty, J. T.: An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, Jour. of. Pharmacol. and Exper. Therap., 1909-1910, i, 579.

[2] Brightman, C. L., Hopfield, J. J., Meacham, M. R. and Acree, S. F.: On the Quinone Phenolate Theory of Indicators. A Spectrophotometric Study of the ''End-points'' and ''Fading'' of Phenolsulphonephthalein Indicators, Jour. Am. Chem. Soc., 1918, xl, 1940.

[3] Lubs, H. A., and Acree, S. F.: On the Sulphonephthalein Series of Indicators and the Quinone-phenolate Theory, Jour. Am. Chem. Soc., 1916, xxxviii, 2772.

LABORATORY METHODS

A NEW AND SHORTER METHOD FOR THE DETERMINATION OF UREA AND SUGAR IN THE BLOOD

By B. Gruskin, M.D.,* Chicago, Ill.

WHILE blood chemistry has developed into a very elaborate branch of biologic chemistry and has expanded until it includes the examination of a considerable variety of blood constituents, it nevertheless remains a fact that sugar and urea represent in a good many, if not in most cases, a sufficient diagnostic guide to the clinician. It seemed, therefore, that it would be desirable to devise a simple, reliable procedure for the determination of these two factors. Rapidity, too, is a very important consideration where the volume of work reaches a high level; as indeed is the case in many a hospital, now that a constantly increasing number of clinicians is appreciating the significance of blood chemistry data.

It is with the aims indicated above that the method offered in this paper was devised. In its favor are claimed increased simplicity and rapidity with undiminished accuracy. The method is a new modification and a unique combination of old principles.

As is well known the estimation of urea, especially in the older methods, has been attended by difficulties of one kind or another; special apparatus, troublesome manipulation or a long lapse of time between the start and end of the determination. Some of the more recent modifications which depend on direct nesslerization of the blood filtrate, after incubation with urease, leave much to be desired as regards the tint and transparency of the solutions to be examined in the colorimeter.

The method in brief is as follows: oxalated blood is incubated with specially prepared urease solution. After incubation a protein free filtrate is prepared. One portion of the filtrate is used for the determination of sugar by the usual Folin-Wu technic and another portion is nesslerized directly for the urea nitrogen. It is necessary to prepare the urease under closely specified conditions so that a minimum amount of bean substance is extracted along with the urease. Foreign bean substance consists of reducing sugars which raise the sugar value and materials which interfere to a greater or lesser extent with nesslerization. However, if the directions given below for the preparation of the urease are reasonably closely followed, no serious difficulty will be encountered.

In no case will the blood sugar be found to be high by more than five or

six milligrams as compared with the regular Folin-Wu method. This is a correction which may be applied if desired; but in the opinion of the author it represents an error which may well be tolerated in routine blood chemistry work, since blood-sugar results are reported as a rule to two significant figures only.

Procedure.—Measure 2 c.c. of blood into a glass tube (6″ × 1″) ; add 2 c.c. of urease solution (for preparation, see below). Mix by agitating slightly and incubate ten minutes at 45° to 50° C. Treat with 14 c.c. of tenth normal sulphuric acid and mix. Add 2 c.c. of 10 per cent sodium tungstate solution and shake; filter. Two c.c. of the filtrate are measured into a Folin-Wu sugar tube and treated in the usual manner.[1] Five c.c. of the filtrate are measured into a tube graduated at 25 c.c. Distilled water is added to make about 12 or 14 c.c. Ten c.c. of Nessler reagent[2] are now introduced and sufficient water to bring the volume up to 25 c.c. This solution is compared in a colorimeter with a standard which contains 0.075 milligrams of ammonia nitrogen and 0.5 c.c. of the urease solution diluted with water and similarly and simultaneously nesslerized. The result is expressed as milligrams of urea nitrogen per 100 c.c. of blood. This figure multiplied by the factor 2.14 gives milligrams of urea per 100 c.c. of blood.

Comments on the Procedure.—It will be observed that manipulation is reduced to the simplest terms. Distillation or aeration of the formed ammonia is eliminated; the variety of reagents is reduced, and there is no need for standard alkali and acid solutions. A buffer solution for the urease incubation is superfluous, the blood being naturally buffered. The amount of filtrate obtained from 2 c.c. of blood is about 11 c.c., so that, where only a limited amount of blood is available, it would be possible to determine urea and sugar on 1.5 c.c. of blood; whereas, for example, a method like Van Slyke and Cullen's requires 3 c.c. of blood for the determination of urea alone.

Attention should be called to the behavior of the blood during precipitation inasmuch as it differs in one important respect from blood treated by the older method. It seems that the alcohol of the urease solution has the effect of causing the mixture to foam. It is obvious then that foaming cannot be taken as an indication of incomplete precipitation of the blood proteins. If it is desired to ascertain this point before commencing the filtration, it is only necessary to allow the precipitation tube to stand quietly for two or three minutes. The precipitate has a tendency to float toward the top and the color of the liquid beneath is pink in cases where the proteins have been incompletely precipitated. The acidity is then adjusted, in the usual manner, by careful addition of normal sulphuric acid.

Preparation of Urease Solution.—Three grams of permutite* are washed in a 250 c.c. Erlenmeyer flask with 100 c.c. of 2 per cent acetic acid and then successively with two 100 c.c. portions of distilled water. Five grams of powdered jack bean meal† and 100 c.c. of 30 per cent aqueous alcohol are now added and the mixture is gently shaken for not more than ten minutes. It is then immediately poured on a suction filter previously prepared with a

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*Central Scientific Company, Chicago.
†Arlington Chemical Co., Yonkers, N. Y.

3-inch Buchner funnel and two layers of No. 2 Whatman filter paper. (The filter should be prepared in advance so as to make it possible to filter immediately after shaking.) If the first 25 c.c. or so of filtrate are turbid they are returned to the filter. The filtrate should be not more than faintly opalescent. The filtrate is finally diluted with its own volume of 30 per cent aqueous alcohol and is kept in the ice box. This urease will keep five or six weeks at ice-box temperature but it is safer not to use a solution that is older than four weeks. As soon as the clear urease solution cools down to approximately 5° C., certain of the more difficultly soluble bean extractives precipitate, thereby rendering the preparation turbid. This turbidity may be disregarded except in the sense that it is necessary to shake before pipetting.

The directions given above are for 150 to 175 c.c. of urease solution. If it is desired to prepare a larger quantity at one time, not only the reagents but the size of the filter, as well, should be increased proportionately. For example, if 450 c.c. of urease solution are to be prepared, instead of 150 c.c., one starts with 9 grams of permutite in a 750 c.c. flask and quantities of bean meal and reagents are trebled throughout. The time of shaking remains the same, ten minutes, and a sufficiently large Buchner funnel is employed so that filtration is complete in two or three minutes. Above all, the combined time of shaking and filtering should not exceed fifteen minutes.

Analytical Data.—Each of a number of blood specimens was divided into three parts. In one part, urea was determined by Van Slyke and Cullen's method. Another part was analyzed for sugar by the regular Folin-Wu pro-

TABLE I

UREA NITROGEN DETERMINATION

Results reported as mg. per 100 c.c. of blood.

SPECIMEN NO.	VAN SLYKE-CULLEN	AUTHOR'S METHOD	DIFFERENCE
1	25 mg.	22 mg.	−3 mg.
2	29 mg.	31 mg.	+2 mg.
3	7 mg.	6 mg.	−1 *
4	22 mg.	22 mg.	0
5	14 mg	13 mg.	−1 mg.
6	19 mg.	18 mg.	−1 mg.
7	80 mg.	82 mg.	+2 mg.
8	43 mg.	45 mg.	+2 mg.
9	18 mg.	16 mg.	+2 mg.
10	16 mg	15 mg.	−1 mg.

*This blood was unusually low in urea nitrogen by both methods.

TABLE II

SUGAR DETERMINATION

SPECIMEN NO.	FOLIN-WU	AUTHOR'S METHOD	DIFFERENCE
1	240 mg.	242 mg.	+2 mg.
2	100 mg.	101 mg.	+1 mg.
3	81 mg.	79 mg.	−2 mg.
4	92 mg.	93 mg.	+1 mg.
5	352 mg.	355 mg.	+3 mg.
6	89 mg.	90 mg.	+1 mg.
7	100 mg.	101 mg.	+1 mg.
8	111 mg.	109 mg.	−2 mg.

Results are expressed as milligrams of sugar per 100 c.c. of blood.

cedure. Finally the third part was analyzed for both sugar and urea by the method described in this paper. The results are compared in Tables I and II.

These discrepancies are not greater than found between any two different methods used in blood chemistry or between duplicate results by the same method.

The sugar values recorded above were reduced by five milligrams to correct for the slight sugar content of the urease solution.

It will be seen that the results are all in pretty fair agreement. Since these comparative tests were made, a large number of blood specimens has been analyzed by this method at my own laboratory and in the Pathological Laboratory of the Mount Sinai Hospital, Chicago, Ill. The results have been uniformly satisfactory in all cases.

Note: Acknowledgment is made to my associate, Dr. Harris, for helping me carry out the experimental work.

REFERENCES

[1]Hawk: Practical Physiological Chemistry, ed. 7, p. 283. Blackiston's Son and Co., Philadelphia.
[2]Idem:

A MODIFICATION OF THE KRAMER-TISDALL METHOD FOR THE DETERMINATION OF SODIUM IN BLOOD SERUM[*]

By Russell L. Haden, M.D., Kansas City, Kansas

THE Kramer-Tisdall method[1] for the determination of sodium in blood serum involves the direct precipitation of the sodium as sodium pyroantimonate, filtration through a Gooch crucible, and weighing. The filtration process has made the method rather uncertain in the hands of other laboratory workers. McVicar and Ross[2] state that: "Even with scrupulous care, we found the method exceedingly temperamental and numerous failures to secure good duplicate results remain unexplained." The technic as originally described requires also the use of platinum dishes for the precipitation, although Wilson[3] has shown that tin dishes may be substituted. The precipitate adheres so closely to glass containers it cannot be quantitatively transferred therefrom.

In attempting to obviate the difficulty and uncertainty of filtration through the Gooch crucible, we first tried fine alundum crucibles as suggested by Dr. Carl H. Green.[4] After a very few filtrations however, the pores of the crucible became clogged and the readings were too high.

We have found that the filtration process may be avoided entirely by the use of the centrifuge and ordinary high form porcelain crucible. A No. 0 crucible will seat well in the top of a 50 c.c. cup of an International centrifuge.

The precipitation is made directly in the weighed crucible, which is then centrifuged. The supernatant fluid is removed by decantation. By utilizing this procedure we have been able to get accurate check determinations and uniformly good results (Table I). All determinations have been made on 1 c.c. of serum since a crucible larger than No. 0 will not seat in the centrifuge cup. The trunnion ring holding a 50 c.c. cup can be reamed out and a larger crucible be set directly in it, if it be deemed desirable to use 2 c.c. of serum.

TABLE I

RECOVERY OF SODIUM FROM KNOWN SOLUTIONS OF SODIUM CHLORIDE

CALCULATED MG. PER 100 C.C.	RECOVERED MG. PER 100 C.C.	ERROR PER CENT
197	193	−2
197	181	−8
246	251	2
246	253	2
296	316	7
296	312	5
346	357	3
346	369	4
394	398	1
394	396	0.5
Aver. 296	302	2

The technic which we have employed is as follows: 1 c.c. of blood serum or plasma is transferred to a weighed No. 0 Coors' high form porcelain crucible. Five c.c. of the potassium pyroantimonate reagent prepared as directed by Kramer and Tisdall is added. Two c.c. of 95 per cent alcohol is then run in drop by drop, the specimen being stirred continuously with a rubber tipped rod. Thorough stirring is very essential. After standing 10 to 15 minutes the crucible is placed in the centrifuge cup and spun for 5 minutes. The speed of the centrifuge should be gradually accelerated. The supernatant fluid is decanted completely, the last drop being removed by touching the rod to the edge of the crucible. Two c.c. of 30 per cent alcohol is then added. This should be allowed to run down the sides of the crucible, care being taken not to disturb the precipitate. The crucible is given a slight rotary motion until a bit of the precipitate spirals up the center. The crucible is again centrifuged and the supernatant fluid removed as before. The washing is repeated, making two washings in all. The crucible is placed in an oven at 110° C. for one hour, allowed to cool in a desiccator and weighed.

CALCULATION.—The weight of the precipitate divided by the factor 0.1108 equals the number of milligrams of sodium per 100 c.c. serum, when 1 c.c. of serum is used for the determination

BIBLIOGRAPHY

[1]Kramer, B., and Tisdall, F. F.: Jour. Biol. Chem., 1921, xlvi, 467.
[2]McVicar, C. S., and Ross, Helen: Jour. Lab. and Clin. Med., 1923, ix, 87.
[3]Wilson, S. J.: Jour. Biol. Chem., 1922, l, 301.
[4]Green, Carl H.: personal communication.

THE USE OF BRILLIANT GREEN-BILE FOR THE COLLECTION AND STUDY OF TYPHOID STOOLS*

By Leon C. Havens, M.D., and Sophie A. Dehler, B.A., Montgomery, Ala.

S PECIAL methods for the isolation of B. typhosus from feces are numerous, and several of them are particularly useful. In all of these, however, attention has been directed to the recovery of the organism from the specimen after it reaches the laboratory, while the value of the specimen has diminished progressively with the time which elapses between its collection and its examination. The specimen must reach the laboratory with the least possible delay to obtain a dependable result, since, with the methods of preserving the specimen usually employed, there is a rapid diminution in the number of viable bacilli of the typhoid-dysentery group.

There are two methods in common use for the collection of such specimens. One is merely to place the feces for the examination in a clean container and start the examination as soon as possible after collection; in other words, have the specimen as fresh as possible. The other method consists in placing the feces in a solution of glycerin (20-30 per cent), which has the advantage of inhibiting B. coli, but also, as will be shown later, has an inhibitory effect on the members of the typhoid group. Consequently it is open to the same objection of increasing unreliability with delay in examination.

An excellent culture medium for typhoid stools would be one which inhibits B. coli and at the same time exerts no deleterious effect upon B. typhosus. The method which is here described accomplishes this result.

DESCRIPTION OF METHOD

Brilliant green is dissolved in bile in a concentration of 1:1500. Difco dehydrated bile is satisfactory and convenient, and has been used in the work described in this paper. After the dye is thoroughly dissolved in the bile it is dispensed into ordinary one ounce wide-mouth bottles, about fifteen c.c. to the bottle. the bottles are stoppered with ordinary corks and sterilized in the autoclave at fifteen pounds for fifteen minutes. After sterilization the mixture keeps indefinitely.

For feces one or two grams are added to the bottle. A larger amount interferes with the action of the bile-dye solution and defeats its purpose.

When the specimen is received at the laboratory a loopful from the surface of the emulsion is plated immediately and this procedure is repeated after twenty-four and forty-eight hours. making three sets of plates from each specimen. The specimen is kept at room temperature in the meantime.

Three platings on successive days are important, because, as will be shown later, the number of positive specimens is doubled by this procedure. The technic after plating follows the common procedure. The cultures here described were streaked on endo agar, suspicious colonies fished to Russell's double sugar agar, and positive cultures agglutinated with known typhoid serum of high dilution (1:50,000).

EXPERIMENTAL

The value of field studies of typhoid is largely dependent upon the value of single feces cultures. Any method which increases the number of positive findings adds to the fruitfulness of carrier surveys and promotes accurate diagnosis of cases. It is known that bile is a good culture medium for the colon-typhoid group and that brilliant green inhibits B. coli. If this dye exerts the same selective action in bile as in broth a concentration might be found which would permit the use of the bile for the multiplication of the typhoid bacilli originally present in the feces and at the same time prevent the enormous overgrowth of B. coli.

Table I shows the effect of varying concentrations of brilliant green in bile upon pure cultures of B. coli and B. typhosus. Bottles containing 15 c.c. of bile in which the dye was dissolved in concentrations from 1:750-1:3000, were inoculated with 0.1 c.c. of broth cultures of these organisms. The bottles were then kept at ordinary room temperature for twenty-four hours, and 0.1 c.c. of the bile-dye solution plated. The table shows the number of colonies after twenty-four hours incubation in one such experiment, which is typical of all the results.

TABLE I

GROWTH OF B. TYPHOSUS AND B. COLI IN PURE CULTURE IN BRILLIANT GREEN-BILE. INCUBATION OF SPECIMENS AT ROOM TEMPERATURE

CONCENTRA- TION OF DYE	P_H 6.0		P_H 6.6		P_H 7.2		P_H 7.8	
	B. TYPHO- SUS:	B. COLI:	B. TYPHO- SUS:	B. COLI:	B. TYPHO- SUS:	B. COLI:	B. TYPHO- SUS:	B. COLI:
1:3000	Innumer- able	5000	Innumer- able	Innumer- able	Innumer- able	Innumer- able	Innumer- able	500
1:2400	Innumer- able	300	Innumer- able	500	Innumer- able	300	Innumer- able	50
1:1800	Innumer- able	100	Innumer- able	300	Innumer- able		Slight in- hibition	
						50	over control	10
1:1500	Innumer- able	100	Innumer- able	100	Innumer- able	15	Slight in- hibition	10
1:1000	2000	200	1500	50	2000	10	1000	0
1:750	60	100	50	20	10	12	15	0
Control Bile without dye.	Innumer- able	Innumer- able	Innumer- able	Innumer- able	Innumer- able	Innumer- able	Innumer- able	Innumer- able

It is seen from Table I that while the selective action of brilliant green on B. coli takes place in bile, much larger amounts of the dye must be used than in broth. Growth of B. coli is inhibited at a concentration of 1:3000 in bile, while inhibition of the typhoid bacillus does not occur until the

dye is used 1:750. The amount of dye used in the method (1:1500) allows a considerable margin of safety on either side, being only one-half the concentration which inhibits B. typhosus and is double that necessary to completely inhibit B. coli in pure culture.

It is further seen from Table I that growth of B. coli is markedly inhibited by all concentrations of brilliant green above 1:3000 at all H-ion concentrations of the bile. But at P_H 7.8 the differential action of the dye appears to be less marked. As one proceeds toward the acid side the inhibitory action on B. coli is less effective. On this basis, we have used the bile-dye solution with the H-ion concentration adjusted between P_H 6.8 to 7.2. If Difco bile is used this adjustment is unnecessary.

Table II indicates an increased inhibitory action of brilliant green in bile on both B. typhosus and B. coli at body temperature. Since the typhoid bacillus grows well at room temperature we have found that better results are obtained by incubating the specimen at 20°-25° C., i.e., in the room, rather than at 37° C.

These experiments, then, indicate that brilliant green dissolved in bile in a concentration of 1:1500 will serve as a useful medium for feces cultures for typhoid. The H-ion concentration should be around P_H 7.0 and the specimen should preferably be allowed to stand at room temperature, because of the apparent increased activity of the dye at 37° C.

TABLE II

GROWTH OF B. TYPHOSUS AND B. COLI IN PURE CULTURE IN BRILLIANT GREEN-BILE INCUBATION OF SPECIMENS AT 37° C.

CONCENTRATION OF DYE	P_H 6.0		P_H 6.6		P_H 7.2		P_H 7.8	
	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:	B. TYPHOSUS:	B. COLI:
1:3000	Innumerable	0	Innumerable	Innumerable	5000	5000	5000	1000
1:2400	Innumerable	3	Innumerable	25	5000	30	2000	200
1:2100	Slight inhibition	0	Innumerable	0	1000	0	5000	10
1:1800	1000	10	Innumerable	50	500	0	1000	50
1:1500	500	5	Slight inhibition	50	100	0	5000	100
1:1250	50	0	500	10	10	0	500	10
Control Bile without dye	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable	Innumerable

These facts were arrived at with the use of pure cultures. In order to determine its effectiveness for typhoid stools, known numbers of B. typhosus were added to specimens of feces received at the laboratory for other examinations. At the same time part of the same specimen of feces containing the same number of typhoid bacilli was introduced into a bottle of 20 per cent glycerin for comparative results.

It is seen in Table III that there is a striking increase in the recovery of B. typhosus from feces in the brilliant green-bile as compared with glycerin

TABLE III

RECOVERY OF KNOWN NUMBERS OF B. TYPHOSUS FROM FECES IN 20 PER CENT GLYCERIN AND BRILLIANT GREEN-BILE

NO. B. TYPHO-SUS ADDED PER GM. FECES	TOTAL SPECIMENS	GLYCERIN 20 PER CENT SPECIMENS POSITIVE				BRILLIANT GREEN-BILE SPECIMENS POSITIVE			
		24 HR.		48 HR.		24 HR.		48 HR.	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
500-2000	19	19	100	9	47	19	100	19	100
50- 100	47	14	30	5	11	37	79	43	91
10- 20	27	2	8	1	4	23	85	24	88
2- 5	7	0	0	0	0	6	85	4	57
TOTAL	100	35	35	15	15	85	85	90	90

solutions. In fact, in six of seven samples, as few as three or four typhoid bacilli were recovered from feces in the bile-dye solution in forty-eight hours, while none could be recovered from glycerin. The experiments summarized in Table III further show that no deterioration of the specimen takes place up to forty-eight hours in the bile-dye solution since the percentage of positive results is slightly higher than at the end of twenty-four hours. The same samples in glycerin, on the other hand, show a progressive decrease in the number of viable typhoid bacilli. The efficiency of the two methods is indicated by the total number of recoveries in each case. With glycerin this was 35 per cent and 15 per cent at the end of twenty-four hours and forty-eight hours respectively, while with the bile-dye solution, it was 85 per cent and 90 per cent. Reference to Table V shows that the typhoid bacillus continues to multiply for seventy-two hours, or longer, since the number of positive results was doubled by plating on the third day.

APPLICATION

It is evident from the foregoing experimental evidence that brilliant green-bile permits the recovery of small numbers of typhoid bacilli from feces. Since the majority of the specimens received at a state laboratory come by mail from considerable distances it is important to have a dependable method which minimizes deterioration due to the age of the specimen. The results obtained with such specimens constitute a severe test of the method. Table IV summarizes these results. Of 241 specimens, typhoid bacilli were isolated from 71 or 29 per cent. These specimens represent single samples from suspected cases of typhoid fever, and from convalescent and chronic carriers. Epidemiologic studies of a number of small rural outbreaks are responsible for the relatively large number of positive carrier cultures.

TABLE IV

RESULTS OF FECES EXAMINATIONS RECEIVED IN BRILLIANT GREEN-BILE

	TOTAL	NO. POSITIVE	PER CENT POSITIVE
Suspected Carriers	126	14	11.1
Suspected cases	115	57	49.5
Total	241	71	29.4

Positive findings in practically one-half of all single specimens sent by mail from suspected cases of typhoid bespeaks a high degree of efficiency for the method used. Since a considerable number of these cases were only suspected to be typhoid more than 50 per cent recoveries were obtained in this series with a single specimen in proved cases.

The results summarized in Tables III and IV give evidence that the usual rapid diminution of typhoid bacilli in feces is prevented by suspension of the specimen in brilliant green-bile. Table V adds further evidence of the truth of this statement.

TABLE V

RESULTS OF EXAMINATIONS OF FIELD SPECIMENS ON SUCCESSIVE DAYS

	24 HOURS	48 HOURS	72 HOURS	TOTAL
Number Positive	34	57	68	71

Table V further shows that if the specimens had been plated only on the day of arrival at the laboratory more than half of the positives would have been missed. If they were to be plated only once the third day is apparently the best time, but it is the routine procedure in this laboratory to plate on three successive days. An increased number of platings, in itself, increases the chances of a positive result.

DISCUSSION

The typhoid bacillus begins to die in feces immediately and, in fact, its numbers progressively diminish in the intestinal tract after leaving the duodenum. This fact has necessitated the use for the recovery of the typhoid bacillus of the fresh feces or of some method of preservation. Successive platings in the laboratory have been valueless for the same reason. A method which permits the organisms of the typhoid group to multiply, not only enhances the value of the initial plating, but permits the use of repeated examinations with increased chances of success. Such a method is especially valuable in a state laboratory where delayed examinations are often necessary. Furthermore the likelihood of obtaining a positive culture from a single specimen is increased, since, even though the bacilli may be few in number in the original sample of feces taken, growth takes place and by the third plating the number of organisms has increased many times. The progressive diminution of the colon bacillus is a further factor in increasing positive results.

The experimental work described in this paper deals with B. typhosus. The method however, has been used for paratyphoid A and B and the dysentery bacillus. with equal success. In other words, the method is useful for all members of this group. The bile-dye solution has also been used with success for blood and urine cultures.

It is needless to point out the value of such a culture medium in field investigations of typhoid outbreaks. Such investigations must frequently be carried out at a considerable distance from a laboratory and it is not always

possible to obtain more than a single specimen from any one individual. Anything which adds to the value of the result of a single specimen is a material aid to the epidemiologist.

In a typhoid carrier survey now being conducted among food handlers the results so far indicate that a rate of approximately 2 per cent will be obtained with this method. The results of this investigation will be made the subject of a future paper.

SUMMARY

A solution of brilliant green in bile will inhibit B. coli and yet allow B. typhosus to multiply. This principle has been utilized successfully in the culture of feces for typhoid. It overcomes the loss of dependability resulting from delayed examination and permits recovery of the typhoid bacillus from the specimen even after several days.

THE USE OF AUTOGENOUS VACCINES IN PULMONARY DISEASES WITH SPECIAL REFERENCE TO THE COHEN-HEIST METHOD OF PREPARATION*

By J. H. CLARK, M.D., PHILADELPHIA, PA.

IT is only within the past ten or twelve years that vaccines have been used to any extent in the treatment of pulmonary infections. Gillett[1] and Pirie[2] in England, and Babcock[3] in this country reported favorably upon the use of autogenous vaccines in chronic bronchitis and asthma. Scheffegrell[4] has employed a mixed treatment with vaccines and pollens in hay-fever with benefit, while Walker,[5] and Hutcheson and Budd,[6] reported favorable results from vaccine therapy in cases of bacterial asthma. Many of the earlier failures were undoubtedly due to the placing of too much reliance on vaccines alone and not considering them as adjuvant to treatment. Within recent years their scope, in pulmonary infections, has increased greatly and many conditions that were formerly treated by "watchful waiting" and unsuccessful surgical methods, have been successfully cured by vaccine therapy.

In a cursory review of recent literature, one is struck with the variety of methods of administration of vaccines, their preparation and the diversity of conditions in which they are used. Minet[7] employed autovaccines in chronic conditions and stock in acute. The sputum was cultured on three successive days, rough estimates of the different organisms present were made and an average of the three days computed. This average formed the basis of the autogenous vaccine. Noting that the bacterial flora in both the acute and chronic conditions was practically constant, he discarded this laborious

method of preparation and later used stock vaccines solely, based on his previous bacteriologic findings. The stock vaccine for chronic conditions consisted of 20 million pneumococci, 40 million tetrageni, 40 million micrococcus catarrhalis and 400 million staphylococci per c.c. This was given every two days, with a rest of five or six days between the fifth and sixth injection, until ten doses in all had been given. In chronic conditions secondary to tuberculosis, he found neither febrile reaction, nor hemoptysis, but noticed a diminution in the cough and improvement in the general health. It was also beneficial in chronic bronchitis. His stock vaccine for acute respiratory conditions was composed of 100 million pneumococci, 50 million streptococci and 350 million staphylococci per c.c. One c.c. was given every two days, dropping to 0.5 c.c. if the heart or kidneys were affected. It is interesting to note that healing was obtained by brutal shock in the acute conditions; if there was no shock there was no healing. This evidently borders on the nonspecific (protein shock) reaction that has been utilized, at times, in typhoid fever and pneumonia. Later[8] he claimed remarkable cures in asthma, using the same stock vaccine for chronic conditions; he again noted a diminution in the quantity and purulent character of the sputum and improvement in the general health of the patients with pulmonary tuberculosis; he cured one-third of his cases of emphysema and catarrhal bronchitis, improved markedly another third and considered an absence of betterment unusual.

The favorable results obtained by Minet influenced other observers to employ stock vaccines in both acute and chronic pulmonary infections. Bourges and Jabard[9] report a case of emphysema and catarrhal bronchitis with recurrent asthmatic attacks of several years standing, in which dyspnea, even on exertion, disappeared after the sixth injection of Minet's stock vaccine, injected every two days. Three months later all auscultatory signs and râles had disappeared and there was no recurrence of asthmatic attacks. These authors also report, as an instance of vaccine therapy, a case of lobar pneumonia, in which 1 c.c. of Minet's stock vaccine for acute respiratory infections was given on the fourth day of the disease. Recovery followed a severe chill, sweat and initial rise of temperature, which later fell to normal and stayed there.

Lambert[10] reports cases illustrating the efficacy of vaccines in suppurative conditions of the pleura. In one, a case of an old purulent pleurisy, with fistula following operation, the cavity was made sterile after ten injections of an autogenous vaccine and closed by secondary suture. A second case of an old revolver wound of the chest, was cured by vaccine therapy alone, although two vaccines were necessary, a procedure that is often neglected in old indolent conditions. DeVernejoul[11] feels that in suppurative conditions of the lungs, surgical treatment should be completed by vaccine therapy and preferably autogenous vaccine therapy.

Abadie and Dufau[12] report a case of abscess of the lung with gangrene, developing after a typical attack of typhus fever, cured by autovaccine. The sputum contained streptococci, staphylococci, Friedländer's bacillus and B. proteus vulgaris. An autogenous vaccine made from these was given at

three day intervals and after the seventh injection the patient was discharged completely cured on the twenty-fourth day.

Dufour and Ferrier[13] emphasize that if vaccines are not efficacious in the early acute case, they may help in those becoming subacute to chronic, confirming their opinion by reporting a case of pleurisy and pulmonary congestion, with fetid sputum, which was cured by autogenous vaccine.

In this day of nonspecific therapy, one hesitates to advocate too strongly the employment of specific bacterial vaccines. Kolmer[14] says, "The treatment of disease by the administration of nonspecific agents has attracted a great deal of attention during the past few years. Indeed, it would appear that this form of therapy is in danger of being overused and its importance over-emphasized." It is especially beneficial in bacterial infections due probably to an increase of congestion about the foci of bacteria and absorption of the bacterial products, leading to an autoimmunization and hence specific stimulation. But does it not seem probable, if nonspecific substances will increase immunity against a definite organism or organisms, by specific stimulation, that specific, definite, bacterial vaccines will be even more efficacious?

Assuming, for the moment, that the use of specific vaccines is just as beneficial as nonspecific, for a certain degree of success in vaccine therapy is probably due to both effects, what type or types of definite bacterial vaccines are considered to be the better, stock or autogenous? For prophylactic purposes stock vaccines are probably to be preferred (Jenkins[15]) or in the treatment of acute pulmonary infections (Minet[16]), but most writers agree that autogenous vaccines, i.e., those prepared from the patient's own bacteria, should be used whenever possible, especially in the vaccine treatment of disease.

Autogenous vaccines to be successful should contain, preferably, the definite and determined organism or organisms, which are responsible for the condition and are not simply saprophytic. It is comparatively simple to grow bacteria from the discharge from an infected area, suspend them in phenolized saline, kill by heating, test for sterility and tell the physician to use so much. But how does one know these are the offending organisms? There are different methods of determining which bacteria are etiologic or pathogenic and which are saprophytic. One method is by agglutination. Walker[17] prepared dried bacterial allergens by repeated centrifuging with saline, alcohol and ether and performed skin sensitization tests with the same. Kolmer[18] employs intracutaneous tests with bacterial suspensions; or one can test the bactericidal activity of the blood by the Lacy-Heist method.[19] All of these methods, however, require more apparatus, simple though it may be and are time-consuming.

Wright[20] working in South Africa on pneumonia, noted that blood flowing from capillaries had bactericidal activity not present in defibrinated, or citrated blood or serum. Cohen and Heist,[21] using the Lacy-Heist method, stated that the bactericidal activity of coagulable blood offered a means of determining natural or acquired immunity. They emphasized[22] the importance of using "unaltered, coagulable, whole, flowing or living blood." Later

Cohen[23] suggested the possibility of separating bacteria, that are etiologic and infecting, from those that are merely saprophytic and nonpathogenic, in copious discharges containing many types of organisms, by growing the infected material directly in whole blood. For in working with Heist and later Borrow, he had determined that, "blood of human beings possesses bactericidal power against large numbers of organisms; that the blood of an individual differs in its bactericidal power against different organisms; that bactericidal power against a particular organism varies in different individuals; that in the discharge from an infected area, organisms can usually be found, against which the blood of the infected person has little or no bactericidal power; that frequently in such discharges or on such area other organisms are found against which the patient's blood has good bactericidal power; that organisms that are supposed to grow well in human blood, fail to grow at all in the blood of some individuals; and that organisms that are supposed to grow poorly or not at all in human blood, may grow with the greatest vigor in the blood of other individuals." It was felt that organisms growing in whole blood was strong presumptive evidence that they were responsible, either as an etiologic or complicating factor, and that when no growth was obtained, they were probably saprophytic. This led to growing the infected material directly in whole blood, plating and identifying the organisms and making a vaccine from the growth so obtained. It had the distinct advantage of saving time and presumably finding the offending organism by a single simple procedure. The method consisted in merely coating the bottom of a sterile test tube with some of the discharge, collecting whole blood aseptically and placing it immediately in the test tube and incubating. Contaminating or nonpathogenic bacteria usually disappeared when grown in the whole blood of the patient studied and as a general rule pure cultures of a single organism were obtained. Cohen and Heist[24] reported several cases of different types of infection successfully treated by autogenous vaccines prepared in this manner, but were conservative in the conclusions drawn, one of which stated, "when an organism present in the respiratory passages, sputum or feces grows in the whole blood of an infected individual, there is a probability that it may be the etiologic or complicating organism."

In our work in connection with the asthma and hay-fever clinic at the Samaritan Hospital, we were disagreeably impressed with the time-consuming method of plating and fishing colonies in preparing vaccines and often disappointed in the results obtained, even though sensitization tests were performed in some cases and vaccines made from the organisms causing positive reactions. The Cohen-Heist method of growing offending bacteria appealed to us as time saving and extremely simple and seemed to do away with the necessity of sensitization tests. In several cases now, we have used the Cohen-Heist method with gratifying results, only a few of which will be reported. In preparing vaccines from sputum we have modified somewhat the original technic. Specimens of sputum are collected after the mouth has been washed out with a mild antiseptic solution. The sputum is then washed in a few changes of sterile saline and a small quantity placed in the bottom of a

sterile test tube. This is covered with about five cubic centimeters of blood freshly drawn from the individual, collected in a sterile syringe with the usual aseptic precautions. The whole is incubated for eighteen to twenty-four hours. No attempt is made at identification, beyond morphology and staining characteristics. The growths obtained have been, with one exception, single pure cultures (Case 3). Nor have there been any instances of contamination with the usual laboratory contaminants.

CASE 1.—Young adult male. Gave a history of having been gassed in the war. Had complained since then of loss of weight, persistent cough and a slightly fetid sputum, moderate in amount. A vaccine was prepared by the Cohen-Heist method, yielding a Streptococcus. Injections were given at four day intervals with only slight local reactions. After five weeks the patient had gained eleven pounds in weight. The disagreeable odor of the sputum had disappeared and cough practically ceased.

CASE 2.—Male aged forty. Following a submucous resection and tonsillectomy, there developed fever, cough and copious fetid foul-smelling sputum, offensive and almost unbearable to patient and family alike. X-ray confirmed the clinical diagnosis of pulmonary abscess. An autogenous vaccine by the Cohen-Heist method gave a large gram positive coccus. Injections were given at four day intervals, with practically no local reaction. After three injections the sputum diminished in amount and lost much of its former offensive odor and the general health improved. A few weeks later a second vaccine was made in the same way from a gram positive coccus. Artificial pneumothorax was performed and a cure effected together with the second autogenous vaccine. The patient has gained forty-five pounds in weight and is back at work.

CASE 3.—Male aged sixty-six. Has suffered for the past twenty-two years with chronic bronchitis and bronchiectasis. Sputum almost one quart daily. Vaccine was attempted in this case solely as a palliative measure to try to diminish the quantity of sputum. The Cohen-Heist method yielded a Streptococcus and Staphylococcus. The sputum has decreased in amount to about a half-pint. The cough during the day has almost entirely disappeared, but there are still paroxysms in the morning until the bronchiectatic cavities have been emptied of the secretion accumulated during the night. The patient has returned to work after six months absence.

CASE 4.—Male aged forty-eight. For the past fifteen years has suffered from chronic bronchitis and asthmatic attacks. The usual sensitization tests had yielded negative results. Culture by the Cohen-Heist method gave a Streptococcus. It is now four months since the vaccine treatment has ended. The cough has entirely disappeared and benzyl benzoate has controlled the asthmatic attacks.

These four cases illustrate the kind of result we have obtained by autogenous vaccine therapy in pulmonary infections. Our experience is not large, nor are all cases entirely successful. We know in certain suppurative conditions, the results obtained by autogenous vaccines are striking, even when used alone. We feel in other conditions, that they form a distinctly beneficial adjuvant to treatment. We submit the Cohen-Heist method of preparation, as it lends itself as a rapid and simple means of preparing an autogenous vaccine, offering, in addition, the detection of organisms against which the individual has no demonstrable immunity.

REFERENCES

[1]Gillett: Brit. Med. Jour., 1913, i, 387.
[2]Pirie: Brit. Med. Jour., 1913, ii, 1268.
[3]Babcock: Jour. Am. Med. Assn., 1915, lxv, 1942.
[4]Scheffegrel: Hay-fever and Asthma, Lea and Febiger, 1922, 225.
[5]Walker: Arch. Int. Med., 1919, xxiii, 220.
[6]Hutcheson and Budd: Am. Jour. Med. Sc., 1918, clv, 826.

[7]Minet, J.: Presse méd., 1921, xxix, 553.

[8]Minet, J.: Lyon Med., 1922, cxxxi, 1107.

[9]Bourges, H. and Jabard, M.: Bull. et mem. Soc. méd. d. Hôp. de Paris, 1922, iii, s. 46, 1402.

[10]Lambert, M. O.: Bull. et mem. Soc. de chir. de Paris, 1921, xlvii, 909.

[11]DeVernejoul: Gaz. d. hôp., 1922, xcv, 95, 245.

[12]Abadie, J. and Dufau: Bull. et mem. Soc. de chir. de Paris, 1921, xlvii, 1279.

[13]Dufour, H. and Ferrier, M. L.: Bull. et mem. Soc. Méd. de hôp. de Paris, 1922, iii, s. 46, 450.

[14]Kolmer: Infection, Immunity and Biologic Therapy, 3rd Ed., 886.

[15]Jenkins, C. E.: Practitioner, London, 1921, cvii, 123.

[16]Minet, J.: J. de méd. et de chir. prat., Paris, 1922, xciii, 751.

[17]Walker: Jour. Med. Research, 1916-17, xxxv, 488.

[18]Kolmer: Infection, Immunity and Biologic Therapy, 3rd Ed., 684.

[19]Heist, G. D., Cohen, S. S., Cohen, M. S.: Jour. Immunology, 1918, iii, 26.

[20]Quoted by Heist, Cohen and Cohen: Tr. Assn. Am. Phys., 1919, xxxix, 109.

[21]Cohen, S. S. and Heist, G. D.: Tr. Coll. Phys., Phila., 1918, 160.

[22]Heist, Cohen and Cohen: Tr. Assn. Am. Phys., Phila., 1919, xxxix, 109.

[23]Cohen, M. S.: New York Med. Jour., 1920, cxii, 967.

[24]Cohen, M. S. and Heist, G. D.: Pennsylvania Med. Jour., October 27, 1921.

THE USE OF ACETYLENE GAS FOR HEMOGLOBIN ESTIMATION*

BY H. R. MILLER, M.D., NEW YORK CITY

IT is well known that carbon monoxide as it passes through fluid blood combines with hemoglobin to form carbon monoxide hemoglobin. Similarly nitric oxide, under the same circumstances, forms nitric oxide hemoglobin. Both these products are stable.

In 1892 Hoppe-Seyler[1] reported a method for hemoglobin estimation based upon the knowledge that hemoglobin and carbon monoxide enter into a permanent union.

In 1901 Haldane[2] revised the method, employing Gower's hemoglobinometer. For a standard he used 1 per cent ox or sheep blood having an oxygen capacity of 18.5 volume per cent, saturating the blood with carbon monoxide. The blood to be tested is laked, bubbled with illuminating gas and matched against a known standard.

Palmer[3] in 1918 further modified the method. Human or ox blood of an oxygen capacity of 18.5 volume per cent, is defibrinated. The oxygen capacity is determined by the Van Slyke method. A 20 per cent solution of blood is made with 0.4 per cent ammonium hydroxide, through which illuminating gas is passed for ten minutes, then carefully sealed and preserved as a standard. To carry out a hemoglobin determination of unknown blood a 1 per cent solution of unknown blood is laked in 0.4 per cent ammonium hydroxide solution, and then saturated with illuminating gas. It is matched in a colorimeter against a standard consisting of a 1 per cent solution freshly prepared from the original 20 per cent standard and newly resaturated with illuminating gas. The standard is set at 10. The calculation is made by dividing the known by the unknown and multiplying by 100. This method is trustworthy and accurate.

Carbon monoxide is not always on hand, especially for bedside studies,

and for many occasions it may be inconvenient or impractical to employ it. I have, therefore, substituted acetylene gas for carbon monoxide. This gas readily unites with hemoglobin to form a combination almost as permanent as carbon monoxide hemoglobin. It has the advantages of being readily produced, of being less toxic and disagreeable when used in ordinary amounts in an open room. I have found it convenient to obtain acetylene gas by dropping a very small piece of calcium carbide (about 50 mg.) into an ordinary 15 c.c. test tube half full of tap water. Gas is generated immediately, the tube is quickly stopped with a rubber plug through which a tube con-
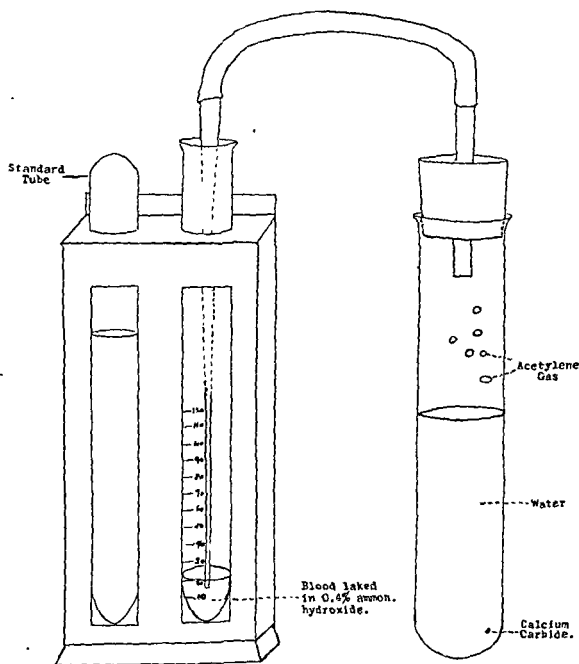
Fig. 1.

nects with a piece of rubber tubing fitted over a narrow capillary pipette (see illustration). The capillary tube may now be led into the solution of blood ready for saturation. A few seconds of bubbling will cause a change to a pink color typical of acetylene hemoglobin.

The use of acetylene gas in hemoglobin estimation has proved practical for the Palmer and Sahli methods. The Palmer method represents a simplification of the Hoppe-Seyler and Haldane principles. Acetylene can be substituted for carbon monoxide. The original 20 per cent standard is prepared with acetylene gas; stored in the dark and on ice it will keep for several

months. As yet we have had no opportunity to test its keeping quality beyond nine weeks.

The use of acetylene gas is also applicable in the estimation of hemoglobin by the Sahli hemoglobinometer. Two millimeters of freshly drawn blood are raised into an accurately gauged Sahli pipette and promptly emptied into 0.4 per cent ammonium hydroxide solution previously poured to the 10 mark into the calibrated Sahli tube. Into this tube acetylene gas is led for a few seconds through a fine capillary pipette, taking care not to raise the fluid by bubbles onto the sides of the tube. Ammonium hydroxide solution (0.4 per cent) is now added drop by drop until the solution is matched in the Sahli instrument against the acetylene treated standard. The standard is prepared by treating 2 mm. of blood of a known oxygen capacity of 18.5 volume per cent with 0.4 per cent ammonium hydroxide, saturating the solution with acetylene gas. This tube is covered with sterile oil, or better, hermetically sealed. A small amount of toluene added to the standard helps preserve it. When not in use it should be kept in the dark and on ice if possible.

REFERENCES

[1]Hoppe-Seyler, F.: Ztschr. f. Physiol. Chem., 1892, xvi, 505-513.
[2]Haldane, J.: Jour. Physiol., 1897-1898, xxii, 298-306.
 Haldane, J.: Jour. Physiol., 1900-1901, xxvi, 497-504.
[3]Palmer, W.: Jour. Biol. Chem., 1918, xxxiii, 119-126.

THE USE OF ULTRA-VIOLET RAYS FOR THE POLYCHROMIZATION OF METHYLENE BLUE*

By Stanley U. Marie and Joseph T. Raleigh, Boston, Mass.

THE ultra-violet ray has been found by us to be very efficient in bringing about the polychroming of methylene blue in connection with Romanowsky stains.

Comparative tests have shown that the preparation of Romanowsky stains, in which the polychroming of methylene blue is required, can be accomplished in much less time by the use of ultra-violet rays than by any of the methods now in use.

The method used by us is as follows: Dissolve 1 gram of methylene blue in 100 c.c. of a 0.5 per cent solution of sodium bicarbonate in distilled water. The mixture is exposed to the rays of any ordinary arc lamp or any other lamp capable of supplying the ultra-violet ray in appreciable quantities for from ten to thirty minutes, depending on the amount and depth of methylene blue mixture, also the type of lamp. A shallow dish five inches in diameter is to be recommended. When the mixture is cool, add slowly without filtering 500 c.c. of a 0.1 per cent yellowish eosin solution until the mixture loses its blue color and becomes purplish with a metallic luster on the surface in which a fine black precipitate is formed. Filter this precipitate on paper and allow it to dry spontaneously. One-tenth of a gram of this dry powder is dissolved in 60 c.c. of acetone free methyl alcohol.

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EDITORIALS

Research

IT falls to the lot of few indeed to make a complete investigation of any problem or to carry it to the uttermost boundaries of its eventual possibilities.

The final solution of scientific problems *de novo et in toto* is more often the result of the work of many than the fruition of the labors of one. Just as a crystal reflecting light from a myriad of facets will vary in brilliance in accordance with the angle from which it is viewed, so will a scientific problem present a varying angle of attack in accordance with the viewpoint of the observer. One carries his work, perhaps, to what appears to be a point of completion; another, following, advances the study still another step. Like the formation of a mosaic, each bit of work ultimately fits in somewhere in the great design which, in essence, is the work of many rather than the expression of the completed purpose of one.

Research, scientific investigation, the patient inquiry and the laborious acquisition of information are often, if one does not pause to think, held to be the prerogative of the professional investigator and to connote a vast expanse of laboratories with their association of equipment, expense budgets and so on.

All these, of course, render the worker's path more easy and his opportunities more accessible but the essential prerequisite for scientific investigation lies in the possession of imagination and the power of acute observation coupled with an infinite capacity for taking pains—which is a definition that someone has given of genius.

Laennec discovered the principle of the stethoscope with no greater equipment than a cardboard tube—but observation and imagination preceded and accompanied his discovery without which, indeed, it would not have been made.

Every man thoroughly and actively interested in the practice of medicine is and should be a potential investigator and research worker. Textbooks of medicine, surgery, diagnosis, therapeutics—what you will—are, in essence, the compiled and correlated individual observations of the multitude. The classical and "typical" descriptions of disease are merely the sum total of a host of "lowly case reports" gathered in serried array; new methods of treatment often have their origin in the imagination of single observers; their final evolution and evaluation is more often the sum total of the labors of many.

The study of carefully kept case records; attention to and cogitation over what in themselves are minutiae with an investigation of what imagination suggests as their possible ultimate significance is just as truly research as an abstruse investigation secluded within the walls of spacious laboratories.

"Theory," says Hippocrates, "is the flower, not the root of experience."

"Put off your imagination as you take off your overcoat when you enter the laboratory," says Claude Bernard, "but put it on again as you do your overcoat when you leave the laboratory. Before the experiment and between whiles, let your imagination wrap you around; put it right away from you during the experiment itself lest it hinder your observant power"—thoughts as applicable to clinical as to laboratory studies.

Every physician should be an investigator, a research worker in essence even if his studies enlighten no one but himself, but if to keen observation and careful thought he couples a capacity for taking infinite pains, he cannot avoid some addition, however minor, to the sum total of medical knowledge.

The clinician and the investigator are one in their object. The labors of one are assisted, corroborated, proved or disproved and finally evaluated by the aid of the other. Clinical and laboratory medicine are not separate entities but inseparable associations and a part of one whole—which is the thought behind the name and *raison d'être* of this journal.—*R. A. K.*

The Evolution of a "Standard" Method of Complement Fixation in Syphilis

AFTER nearly twenty years of painstaking effort the complement-fixation test has come to occupy a definite and fairly well defined place in the clinical and laboratory study of syphilis and, in the interest of uniform and strictly comparable results, the attention of serologists and clinicians is now focussed upon the problem of evolving a technic acceptable for uniform adoption.

The problem has been studied by various investigators and groups from various angles but while much has been done and various methods of performing the test proposed, their general adoption or even wide-spread trial has not followed for reasons which need not be elaborated.

It is rather apparent that, to secure its general adoption, the standard method, if it be not the joint product of a delegated group, must have, to secure its general trial and eventual adoption, the accolade of approval officially or semi-officially conferred upon it in so unmistakable a manner that it will be impossible for the conscientious worker to ignore it on any grounds.

Service or cooperation with such a group engaged in such a problem should be considered a public duty and there already exist two representative bodies of laboratory and clinical workers preeminently fitted to conduct such a survey, one of which has already manifested a collective interest in the problem: The American Public Health Association, and the American Society of Clinical Pathologists.

The personnel of either—or better, of both—of these organizations furnishes an unimpeachable forum for the presentation and discussion of ideas and a representative body by which investigations may be conducted. If a consensus of agreement can be reached by either or both in conjunction, its tenor will be sufficiently representative and authoritative to cause it to be heard and respected.

In order to avoid unnecessary repetition of completed investigations whose accuracy and significance is accepted, an important preliminary to such an investigation, it might be suggested, would be the collection and analysis of opinion as to the essential desiderata to be incorporated in a standard technic and from which the specifications of such a technic could be constructed.

Next in order, because of the difficulty of securing a general acceptance of methods with which a certain proportion of workers may be more or less unfamiliar and in favor of which, accordingly, they are apt to be reluctant to cast aside those well tried by time and personal experience; and in order to avoid the impression of personal bias in the investigation it would seem logical as well as feasible to select from those methods in the field at large, those which claim or appear to most nearly approximate the requirements for a standard technic.

By apportioning these for comparative trial among the personnel of the investigating bodies it should then be easy to secure within a short space of time a sufficiently large number of comparative tests without unduly burdening any worker or laboratory.

One thousand tests by one hundred workers gives a total of one hundred thousand reactions for comparison and analysis in much less time than would be possible for one and, in addition, the personal factor is minimized in their analysis.

By the same method when, at any time, an apparently irreconcilable difference of opinion arises in the consideration of essential desiderata, its thorough comparative investigation is rapidly attained.

If, at the end of such a comparative trial, the field can be narrowed down to one or two or three methods which excel preeminently, the further investigation may be concentrated upon these in the endeavor to see which is the most satisfactory and adaptable to the standard specifications; if none, then the construction of a new technic is indicated and the pathway toward it cleared of many obstacles.

Coincidently with the conduct of such an investigation and analytic survey of the whole, a similar collective study of the parts may be made with reference to the acceptable standard for reagents and so on.

It is much more likely that the *adoption* of such a proposed standard method—which, after all, is the crux of the problem—would more rapidly follow such a collective and authoritative report, than as a sequence to the work of individuals or individual groups.

Even if such a collective investigation should fail at the first attempt, to evolve a perfectly satisfactory and acceptable standard method, it could not fail to finally rid the field of its present impedimenta · of loosely constructed methods which, it must be admitted, abound in general practice.

It is not to be assumed, nor would it be tolerated, that any method by whomsoever proposed, should constitute the final and irrevocable solution of the problems inherent in complement-fixation tests. Investigations as to the mechanism and significance of this phenomenon should and would, undoubtedly, continue but their results should be more readily approximated for study and analysis when the methods whereby they were obtained are more strictly comparable than they are at present.   —R. A. K.

The Gorgas Memorial

DURING the past year, throughout the United States, the work of organizing the Gorgas Memorial State Governing Committees has been progressing. In some states the response has been most enthusiastic, while in others considerable effort has been necessary to bring home to the doctors the importance of this movement to them, individually and collectively. Inasmuch as the Gorgas Memorial is primarily a medical movement and as such must have the united support of the profession if it is to make the proper impression on the general public, we take this occasion to outline briefly the Gorgas plan and to request the cooperation of our colleagues in bringing to a successful issue this national health program.

We are planning to establish a memorial for our former chief, Major General William Crawford Gorgas, not of marble or bronze, but a permanent .

living organization in the form of a great health foundation typical of his work in research and curative medicine, that will unite laymen and doctors in an intelligent effort to obtain better personal health—a health guild that will be supported and directed by the representatives of curative medicine.

The Gorgas Memorial consists of two phases:

1. An Institute in Panama for research in tropical diseases.
2. A health educational program in the United States and other countries that wish to cooperate and participate in the movement.

We are living in an age when people are knocking at all doors of knowledge and demanding that they be admitted. In the field of medicine who are so well fitted to meet this demand as those actually engaged in the practice of medicine? The doctors have a far more interesting and important message to deliver than any other group.

In the United States today there is scarcely a community that has not its quota of irregular "medical practitioners," so-called. In many states there are strong organizations of the representatives of the various cults, whose theories are imposed upon an uninformed public. Public ignorance is encouraged by professional reticence and the result is the astounding growth of unscientific methods. If the profession is to maintain the high standing to which centuries of labor in behalf of suffering mankind entitles it, it is essential that a definite organized effort be made to familiarize the public with such facts as will impress upon it the importance of medicine's contributions to human welfare. A constant fund of proper health information through the newspapers, magazines, lectures, moving pictures and the radio, furnished by medical men and women of known reputation and standing, will direct the public to the proper source for medical advice and gradually eliminate the irregular practices constantly increasing.

One of the objects of the Gorgas Memorial is to furnish a channel through which this kind of information may be disseminated. It cannot be done by individual physicians. It must be conducted by a dignified, ethical organization, controlled by the medical profession. The name of Gorgas is synonymous with "better health." No more appropriate name could be adopted for a movement that has for its object, *the development of cooperation between the public and scientific medicine for the purpose of improving health conditions by implanting the idea in the mind of every individual that scientific medicine is the real authority in all health matters and as such should be recognized as the source of health instruction.*

Before we ask the public for financial and moral support, it is essential that the doctors of the country unite in support of this program. As a means to this end, Governing Committees are now in process of organization, on the basis of 100 members to every 1,000,000 population in each state. Seventy-five per cent of the personnel of each committee will consist of medical men and 25 per cent of influential laymen and women. The permanent activities of the organization will be supervised by these committees in their respective states, in cooperation with the National Executive Committees.

An organization cannot operate without funds. We are endeavoring to raise an endowment of $5,000,000, the interest only of which will be utilized to carry on the work. The principal will be invested in trust securities and remain intact. None of the money thus obtained will be spent for buildings or equipment. The Republic of Panama has donated the site and guaranteed the initial buildings and equipment for the tropical research laboratories, in recognition of Gorgas' great work in Panama. Those invited to serve as Founder members of the State Governing Committees are requested, as they accept membership on the Committee, to subscribe $100 to the Endowment Fund, payable within two years. Every individual on the State Committee is a contributing member. When the medical nucleus of the organization is complete, a general appeal for funds will be made to the public.

The American Medical Association, at its recent meeting in Chicago, passed the following resolution:

> "RESOLVED, That the House of Delegates of the American Medical Association, convinced of the great promise which the Gorgas Memorial contains of benefit to humanity through improved knowledge of preventive medicine and tropical disease, and of its peculiar adequacy, as a tribute to our great leader and sanitarian, recommend to the organized profession of the country, through its constituent state and county societies, the enthusiastic support of the project."

<div align="right">

J. A. Witherspoon, Tennessee,
Joseph Rilus Eastman, Indiana,
Thomas Cullen, Maryland,
W. H. Mayer, Pennsylvania,
F. B. Lund, Massachusetts.

</div>

The Memorial has also been endorsed by numerous other medical and civic organizations.

Every doctor is requested to take a personal interest in the Gorgas program and to see that his community is adequately represented on the State Governing Committee. Each County Society should appoint officially at least one of its members to serve on the State Committee. This is one foundation that is controlled by the practitioners of curative medicine and as such should be supported by every practicing physician. Let us pull together, "the doctor for the doctor."

Frank Billings
Gilbert Fitz-Patrick
Seale Harris
W. H. G. Logan
Samuel J. Mixter
G. H. de Schweinitz
Rear Admiral E. R. Stitt
George Crile
William D. Haggard
Franklin Martin

William J. Mayo
Stuart McGuire
Ernst A. Sommer
Ray Lyman Wilbur
Surgeon General Hugh S. Cumming
C. Jeff Miller
Major General Merritte W. Ireland
Brigadier General ·Robert E. Noble
George David Stewart
Hugh Young

Medical Members, Board of Directors,
Gorgas Memorial Institute,
Executive Offices: Chicago, Illinois.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

How Our Bodies are Made*

THIS surprisingly attractive work on elementary physiology is written, as the title would suggest for "the young idea," when at the age of six or seven it commences to bombard us with questions as to how our bodies are made. The illustrations are readily understood by the age for which the book was written. Thus we see airplanes sailing in through the mouth and moving downward through the air passages into the lungs, where their cargoes are transferred into submarines in the blood and carried through the circulatory channels to remote parts of the body. Or again sailing vessels travelling downward through the esophagus discharging their cargoes at wharves in the intestines where they are met by accommodation trains which travel back through the route of the lymphatics. Even the delicate question as to how people are born is treated in this novel manner, with what the reviewer considers fair success. Bacteria, we find, are black demons with javelins and arches. The defenses of the body are represented by armored soldiers and by the white submarines of the blood. The bodies of these black demons which have been destroyed are placed in the submarines and carried away.

The central nervous system is, of course, represented by a telephone system and is explained in terms of photography, etc. Although there is some risk of overemphasizing the analogies, we feel that in view of the purpose for which the book was written it has been admirably done and will answer satisfactorily the questions proposed by youngsters of early school age.

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*How Our Bodies are Made: By R. M. Wilson, M.B., Ch.B. Cloth. Pp. 246. Illustrated. Henry Frowde and Hodder & Stoughton. London.

The American Society of Clinical Pathologists

Dr. John A. Kolmer, Philadelphia, Pa.
President

Dr. Frederick E. Sondern, New York
1st Vice President

Dr. W. F. Thomson, Beaumont, Texas
2nd Vice President

Dr. Ward Burdick, Denver, Colorado
Sec'y-Treas.

Service Bureau

The American Society of Clinical Pathologists maintains a Service Bureau for the benefit of its members which receives requests from hospitals and other institutions desiring the services of a clinical Pathologist. Any member wishing to change his location should communicate with the Secretary.

Eighth Semi-Annual Meeting of the State Pathological Society of Texas

Our colleagues of Texas constitute, in Main Street parlance, a live wire organization very active in promoting the interests of clinical pathology. The State Pathological Society of Texas is a pioneer in the field and has recently held its eighth semi-annual meeting.

The proceedings of their last reunion at Houston, October 8, 1924 covered the following interesting program:

PROGRAM

1. President's Opening Address
   Dr. B. F. Stout, San Antonio, President

2. The Value of Differential Leukocyte Count in Diagnosis. (Lantern Slide Demonstration)
   Dr. M. D. Levy..............Houston

3. The Surgeon and the Pathologist
   Dr. John T. Moore...........Houston

4. Primary Round Cell Sarcoma of the Heart, with Report of Case
   Dr. W. H. Hill and
   Dr. Henry Hartman......Galveston

5. Is the Pathologist Properly Appreciated?
   Dr. M. F. Bledsoe........Port Arthur
   President Texas State Medical Association

6. The Last of the Pathologist
   Dr. E. F. Cooke.............Houston

7. Discussion of the Pathological Problems of Today
   Opened by Dr. A. H. Braden..Houston

8. Dinner at San Jacinto Inn

CLINICAL AND EXPERIMENTAL

A STUDY OF THE EFFICACY OF CERTAIN ANTIDOTES IN THE
TREATMENT OF ACUTE BICHLORIDE OF MERCURY POISONING*

By W. H. Zeigler, Phar.D., Charleston, S. C.

DURING the past ten or fifteen years a great deal of time has been spent by many investigators in an attempt to establish a reliable treatment for acute bichloride of mercury poisoning. Rabbits and dogs were the animals used in most of the experiments.

After a careful review of the literature on the subject, the following facts seem to be established: First, that clinically we have had both recovery and death from toxic doses of mercuric chloride; second, that when certain amounts of mercuric chloride are administered by mouth or subcutaneously to animals, death may or may not occur; third, that the antidotes which have been proposed by various investigators, who have obtained beneficial results with them, have been found worthless in the hands of others.

Egg albumen and milk is the most commonly used antidote in bichloride of mercury poisoning. It is often used regardless of whether the poison has been absorbed or not. We must remember, however, that albuminate of mercury is soluble in the acid of the gastric juice and when albumen is administered, it should be removed immediately. Fantus[1] recommends a mixture of sodium hypophosphite and hydrogen peroxide; Fantus and Hyatt[2] found Carter's antidote, which is a mixture of potassium and sodium acetate, effective, if administered within five minutes after the poison had been taken; Sabbatani[3] recommends hydrogen sulphide; Hall[4] a mixture of potassium iodide, quinine and dilute hydrochloric acid; Wilms[5] reported an effective chemical antidote in calcium sulphide dissolved in sterile water and filtered, which he

recommends to be administered intravenously, one grain of calcium sulphide given for each grain of bichloride of mercury taken. His experiments consisted in administering to dogs, one bichloride tablet and following it some time afterward by the antidote both by mouth and vein. He also reports a number of clinical observations. This antidote has been used since then with apparent success by several practitioners. Dr. E. T. Kelly, of Kingstree, South Carolina, reports a case of bichloride of mercury poisoning, in which Wilm's antidote was used with success, although the treatment was not begun until forty-eight hours after the poison was taken and four-plus albumin had appeared in the urine. Haskell and Courtney,[6] injecting subcutaneously into dogs, bichloride of mercury in fifteen mg. per kg. doses, found calcium sulphide to be of no value and gave as their opinion, that "this antidote is fraught with actual danger and it is possible that cases of bichloride poisoning may have the lethal exitus hastened rather than retarded by this drug."

Realizing the difficulty of producing a chemical detoxication in the blood stream, Burmester[7] recommended venesection and transfusion. MacNider,[8] in a series of experiments made upon dogs in which he used uranium nitrate, found "that the severity of epithelial damage to the kidney shows a correlation with the degree of acid intoxication produced by the metal, and furthermore, that the damage may, in large measure, be prevented by the intravenous injection of an alkaline solution," therefore, the use of alkalies in acute bichloride of mercury poisoning seems to be justified; Lewis and Rivers[9] used glucose and sodium bicarbonate by mouth and vein to combat acidosis. Sansum[10] found the minimum lethal intravenous dose of bichloride of mercury for dogs to be 4 mg. per kg., and, experimenting with both Carter's and Hall's antidotes, came to the conclusion that they were worthless.

Barbour[11] in a series of test tube experiments, in which he used bichloride of mercury and fresh ox serum in varying concentrations to which he added Hall's antidote, found that no precipitate occurred in concentrations as low as 1-1000. His conclusions were that this antidote was worthless because the poison has a greater affinity for the tissue than for any nontoxic remedy.

Burmester and McNally[12] administered by mouth .024 to .5 gm. of bichloride of mercury per kg. to dogs, and found that the mercury could be detected chemically in the blood of the animals in a very few minutes after its administration; that in massive intoxication, immediate renal changes, varying with the size of the dose, occurred instantly.

Haskell and coworkers[13] in a series of experiments in which they administered to dogs 10 mg. of bichloride of mercury per kg. by mouth found that the intravenous injection of twenty-five cubic centimeters of eight-tenths per cent saline solution per kg. possessed a definitely beneficial action and effects the recovery of animals by preventing absorption or hastening elimination.

Lambert and Patterson's[14] method has been used with apparent success. This form of treatment is especially used in northern hospitals. They recommend the whites of several eggs and the washing out of the stomach,

also the testing of the stomach contents, urine and feces, for mercury. As soon as the nausea is allayed the following routine:

1. The patient is given every other hour eight ounces of the following mixture: Potassium bitartrate, 1 dram; sugar, 1 dram; lactose, ½ ounce; lemon juice, 1 ounce; boiled water, 16 ounces. Eight ounces of milk are administered every alternate hour.

2. The drop method of rectal irrigation with a solution of potassium acetate, a dram to the pint is given continuously.

3. The stomach is washed out twice daily.

4. The colon is irrigated twice daily, in order to wash out whatever poison has been eliminated in that way.

5. The patient is given a daily sweat in a hot pack. They stress the importance of keeping up the treatment with the colonic drip enteroclysis day and night without interruption.

DISCUSSION OF EXPERIMENTS

This investigation was the result of several inquiries made by physicians as to the value of calcium sulphide as an antidote when administered both by mouth and intravenously.

Although both Haskell and Courtney,[6] and Wilms[5] had made reports on this antidote, the author felt justified in conducting a series of experiments in which all possible errors, in regard to the amount of absorption, would be eliminated, by injecting the poison directly into the circulation.

After placing a tourniquet on the lower part of the thigh, the poison and the antidote having been dissolved in sterile normal saline solution, were injected into a branch of the vena saphena parva on the lateral face of the leg. The injections were made through a hypodermic needle which was connected to a 50 c.c. burette by a rubber tube. In order to check up results of other investigators, the poison was also administered by mouth.

Fifty dogs and several rabbits were used in this research. The animals were divided into five groups.

Group No. 1. Was given one-half of a bichloride of mercury tablet (about 0.2 gm. or 3.5 grains) by mouth No antidote was given in this series.

Group No. 2. Was given calcium sulphide in aqueous solution by mouth.

Group No. 3. Was treated as in group one and then given almost immediately calcium sulphide, an equal amount in aqueous solution by mouth.

Group No. 4. Was given 4 mg. of bichloride of mercury per kg.; the dose being given intravenously.

Group No. 5. Was treated as group four and then an equal amount of calcium sulphide was administered both by mouth and intravenously.

The dogs in groups one and three were given colored bichloride of mercury tablets, so that the vomitus could be watched. The disintegration of the tablet undoubtedly takes place very rapidly, because in no case was any part of the tablet vomited, although the vomitus was colored. Wilms[5] and others used one bichloride tablet by mouth; the author found that one-half of a tablet placed on the back of the tongue and washed down with water was sufficient to produce death.

Group two was given from three to seven and one-half grains of calcium sulphide to which from 50 to 100 c.c. of water had been added. The only effect noticed was that vomiting would occur unless the drug was well diluted. A solution was also made by boiling the calcium sulphide with distilled water and filtering. The filtrate was injected intravenously into both rabbits and dogs. It was found that unless a 1-1000 solution was used, and injected slowly, marked asphyxial symptoms developed; the animal dying in convulsions. On account of the insolubility of calcium sulphide, an attempt was made throughout these experiments to use sodium sulphide which is freely soluble. In the test tube both of these salts changed the mercuric chloride into the insoluble sulphide very rapidly.

The third group of dogs was given one-half of a bichloride tablet (about three and one-half grains) by mouth, followed by either sodium or calcium sulphide dissolved in 100 c.c. of water. It was found that unless this antidote is administered within three minutes after the poison it is worthless and may, if corrosion has taken place, actually hasten death. In several instances, when the antidote was administered some time after the gastro-intestinal symptoms had developed the animals died immediately; death evidently being due to shock.

It is the opinion of the author, that if administered immediately after the poison, calcium sulphide dissolved in water is of more value than egg albumen.

Knowing that colloids would delay the absorption of alkaloids, an attempt was made to determine if mucilage of acacia would not by adsorption lessen the corrosive action of the bichloride of mercury.

A series of test tube experiments were made in which bichloride of mercury was precipitated from an aqueous solution and simultaneously from an acacia solution; the percentage used being three per cent potassium iodide solution. Into test tube "A" 15 c.c. of a 1-1000 bichloride of mercury solution was placed, and six drops of 3 per cent potassium iodide were added. The usual red precipitate was the result. Then into test tube "B" 7½ c.c. of a 1-500 bichloride of mercury solution was placed. To this 7½ c.c. of 35 per cent solution of mucilage of acacia was added; thus diluting the bichloride to 1-1000. Upon addition of six drops of 3 per cent potassium iodide solution, a distinctly yellow precipitate was the result.

These experiments are of interest, and can only be explained on the theory that by adsorption the mucilage of acacia prevents the action of the potassium iodide on the mercury. This indicates that similar results should be obtained in the stomach and intestines; that is, because of the mucilage the mercury would be prevented from attacking the mucosa of the stomach and intestine. Several preliminary experiments were made to determine whether or not this theory was true.

Three and one-half grains of bichloride of mercury dissolved in 200 c.c. of U.S.P. mucilage of acacia was administered by stomach tube. The only signs of the corrosive action of the mercury was the vomiting that occurred about five minutes after the administration. The vomitus was tested for mercury, the test proving negative. One hundred c.c. of mucilage

of acacia and 30 c.c. of 25 per cent magnesium sulphate was given four hours after the poison. The animals survived.

Several animals were also given intravenously, a total of 65 mg. of mercuric chloride, followed by an intravenous injection of 5 per cent acacia in nine-tenths per cent salt solution. It was thought that since the corrosive effect is lessened in the gastrointestinal canal, when administered by mouth it would also lessen the corrosive effect upon the stomach, intestine and kidney, during the process of elimination. The animals died within twenty-four hours without showing any gastrointestinal symptoms, although when posted the mucous membrane of the stomach and intestines were found covered with mucus and the mucosa easily detached. It is very evident, from these preliminary tests, that mucilage of acacia is of value, when given by mouth, to prevent absorption and to allay inflammatory conditions. By vein it is contraindicated after absorption of the mercury.

Since Sansum[10] had established a minimum lethal intravenous dose of 4 mg. per kg. for the dog, this dose was injected into a branch of the saphenous vein of the dogs of group four, and the animals closely watched. It was observed that this amount did not always produce death, although the gastrointestinal symptoms were always present. An attempt was made then, to determine if the weight of the dog was not a factor. Several puppies and small dogs were injected in this manner, and it was found, that unless the total amount was forty or more mg., it did not produce death.

Group five was treated as in group four and followed, at times, immediately afterwards by an equal amount of either sodium or calcium sulphide, dissolved in sterile water. The antidote was ineffective. A solution of iodide of soda was also injected without effect. It is very evident to the author, from a close observation of these experiments, that when a sufficient quantity of bichloride of mercury is absorbed into the blood stream, that we have no means of combating the corrosive effect upon the mucosa of the intestine or the epithelial cells of the kidney. The animals posted in groups four and five showed marked corrosion of the walls of the small intestine, which is a sufficient cause for death, regardless of the effect upon the kidneys.

It is the opinion of many clinicians that when a toxic dose of bichloride of mercury is taken, unless treatment is administered almost immediately, death will take place eventually. That is, if the poison has been absorbed, it will produce nephritis and death. MacNider and others have made extensive studies of the effects of bichloride of mercury on the cells of the kidney and liver and undoubtedly this effect is quite a factor in producing death, but from the observations of the author in this series of experiments, it appears that the corrosive effect on the mucosa of the stomach and intestines both before and after absorption is the most important factor in the cause of death. My opinion is based on the postmortems which show conclusively that bichloride of mercury produces marked pathogenic lesions in the small intestine. Mercury seems to have a peculiar affinity for the mucous membrane of the stomach and upper intestine. This was demonstrated also by the intravenous injection of toxic doses. After administering the drug in this manner the animals developed gastrointestinal symptoms; i.e.,

bloody stools and vomitus and, when posted, evidence of the corrosive action of the drug, as it was eliminated into the intestine, was found.

The report by Lambert and Patterson,[14] of a case in which one and one-half gr. of bichloride of mercury was injected intravenously into a patient by mistake, is interesting in that it coincides with the observations made upon the animals of group four. "Eight hours after the injection of the drug, the patient vomited and the pulse became irregular and weak. The bowels began to move involuntarily and continuously and were bloody and full of mucus. Later there was almost total suppression of urine and the patient died in extreme exhaustion, five and one-half days after the injection." The essentials of the postmortem were as follows: "The kidneys were very large and deeply jaundiced. The capsule stripped with some difficulty, leaving the surface roughish. The mucous membrane of the stomach was very much swollen, very red and covered with a layer of thick mucus. The intestines were filled with a blackish watery feces, mixed with a large amount of mucus. The descending portion of the colon and sigmoid were covered with membrane and deeply congested. The lesions were most severe in the sigmoid. The anatomic diagnosis was fixed as: Hemorrhage into the lesser peritoneal sac and gastric ligaments, chronic diffuse nephritis with acute degeneration." I believe that if as much as one-fifth of a bichloride of mercury tablet (1.4 gr. or 90 mg.) is absorbed, death is inevitable.

From the experiments conducted in this research it appeared that unless corrosion took place the animal did not succumb, although quantities of albumin could be found in the urine. It was observed also that after the animal showed signs of great depression, the mere attempt to insert a stomach tube would result in death. And when an antidote which was slightly alkaline was administered in this manner, the animal would immediately die. Knowing then, the corrosive effects of the drug, both before and after absorption, a great many experiments have been made in an attempt to find some agent which would lessen this effect by changing the mercury into a less irritant form.

I believe that the recoveries that have taken place, when this or any other antidote has been used, would have occurred as well without it. It was noticed that when nontoxic doses were administered albumin regularly appeared in the urine but disappeared after a time without treatment. This acute nephritic effect is one that can also be produced by uranium and other irritant drugs.

CONCLUSIONS

1. That the minimum lethal oral or intravenous dose of bichloride of mercury for the dog cannot be based upon the weight of the animal.

2. That the lethal oral dose of bichloride of mercury for the dog is 200 mg. (about three gr.).

3. The minimum lethal intravenous dose of bichloride of mercury for the dog is 40 mg.

4. That calcium and sodium sulphides, dissolved in water and administered by mouth grain for grain, are effective antidotes for acute bichloride

of mercury poisoning, if administered before gastrointestinal symptoms have occurred.

5. That when a total dose of 40 mg. of bichloride of mercury is injected intravenously, calcium sulphide, sodium sulphide or iodide of soda is of no value as an antidote administered either orally or intravenously.

6. Mucilage of acacia will prevent the corrosive action of bichloride of mercury and delay the absorption.

TREATMENT RECOMMENDED

The author recommends the following treatment in bichloride of mercury poisoning; the procedure depending upon the amount of corrosion produced by the drug.

If the patient is seen immediately after taking the bichloride of mercury, undoubtedly the washing out of the stomach with a 1-1000 unfiltered solution of calcium sulphide is the best treatment. This should be followed by the administration of about 300 c.c. of mucilage of acacia U.S.P. If vomiting has already occurred, which is the first effect of the corrosive action of the drug, the same treatment would be used, but if other gastrointestinal symptoms are present, calcium sulphide is contraindicated. The stomach should be washed out with care and quantities of mucilage of acacia given both by mouth and by high colonic injection. A mild cathartic should also be given. If the patient develops anuria, efforts should be made to restore this function. A mixture of acetate of potash, caffein citrate and infusion of digitalis should be given every four hours for several days.

Other well-known methods of elimination should be used. Mercury is absorbed as soluble organic compounds of proteins and is deposited in various organs of the body. Sollman[15] says, after long continued administration, the excretion persists intermittently for many months. It is excreted mainly in the urine and feces. It is, undoubtedly, also secreted by the stomach. Lambert and Patterson[14] recommend testing, at regular intervals, the stomach contents, urine and feces, for mercury. As the whole treatment depends upon the absorption and elimination, this is very necessary. As long as the mercury is found in the secretions, the diuretic mixture should be continued. Three hundred c.c. of mucilage of acacia should also be given at least once a day by mouth and 100 c.c. by high colonic injection. The mucilage will prevent the reabsorption of the mercury from the stomach and intestines. There are several tests for mercury. Klein's is considered very delicate. Add a little KI, a drop of ammonium chloride and then NaOH drop by drop: brown or yellow color or precipitate (NHg_2I).

Finally, the author does not believe that a satisfactory antidote has been found which when injected intravenously will change the mercury into a less corrosive salt. The whole treatment of acute mercury poisoning should be aimed against absorption, to prevent the local effect, and to increase as rapidly as possible the elimination and prevent the reabsorption of the drug.

The author wishes to express his appreciation of the valuable assistance of the following members of the faculty: Dr. Francis B. Johnson of the

Department of Clinical Pathology, Dr. Henry Plowden of the Department of Pathology, Dr. H. Grady Callison and Mr. E. C. Hood of the Department of Pharmacology.

In the following, additional detail is given in regard to some of the experiments.

GROUP 1.

Dog No. 52. Weight 5 Kg.

First day, 11.57 A. M. One-half bichloride of mercury tablet (about three and one-half grains) followed by 100 c.c. of water, given by mouth.

12.02 P. M. Dog vomits, vomitus shows bluish color due to color of tablet.

3.30 P. M. Dog living but appears to be in shock.

Second day, Dog died during the night.

GROUP 4.

Dog. No. 17. Weight 10.6 Kg.

First day, 5. P. M. Drug: Bichloride of mercury, 4 mg. per kg. intravenously. The poison was dissolved in .9 per cent salt solution and injected into a branch of the saphenous vein. Total dose 42.4 mg.

5.10 P. M. Dog vomits.

Second day, 10. A. M. Bloody feces noted in cage.

4. P. M. Passes urine.

Third day, 10. A. M. Specimen of urine collected and examined. Contaminated with feces, containing a quantity of blood.

Fourth day, 10. A. M. No improvement.

Fifth day, Dog died during night.

GROUP 5.

Dog. No. 21. Weight 7.5 Kg.

First day, 5.40 P. M. Drug: Bichloride of mercury, 4 mg. per kg. intravenously. The poison was dissolved in .9 per cent salt solution and injected into a branch of the saphenous vein.

Second day, 10.30 A. M. Profuse defecation, showing blood. Evidences of vomiting during night.

1. P. M. Calcium sulphide, 30 mg. dissolved in distilled water after boiling for 15 minutes, filtered, and injected into a branch of saphenous vein.

3.45 P. M. 65 mg. calcium sulphide orally, vomiting occurring immediately.

5. P. M. Dog weak, respiration shallow, heart rate rapid.

Third day, Dog died during night.

Post: Liver apparently normal, intestines markedly corroded. Mucosa strips easily from serous coat. Hemorrhagic spots with apparent perforations present in walls of small intestine. Kidneys swollen, dark purplish in color, showing areas of hemorrhage.

SPECIAL EXPERIMENT.

Dog No. 29, (Small Puppy). Weight 1.5 Kg.

First day, 11.50 P. M. Drug: Bichloride of mercury, 4 mg. per kg. intravenously. The poison was dissolved in .9 per cent salt solution and injected into a branch of the saphenous vein. Total dose: 6 mg.

12.40 P. M. Small greenish stool noted.

Second day, Animal fussy.

Third day, No change.

Fourth day, Animal lively. Site of injection sloughed slightly. Cleaned and dressed with ichthyol ointment.

Fifth day, Animal seems weak.

Sixth day, No change.

Seventh day,   Animal vomiting at intervals, passing worms.
Eighth day.   Improved.
One month after,   Alive, legs much improved.

NOTE: Since the above article was received for publication I concurred with Dr. Leland B. Salters, of the Florence Infirmary, who tried the treatment recommended, with success. He writes, "I want to thank you for the information given in this case because without it I do not know just what course I would have taken."

The patient, aged twenty-three, white, male, single, following a period of whiskey drinking, swallowed three seven-and-one-half gr. bichloride of mercury tablets on Monday, June 9, 1924, at eleven A. M. Treatment was commenced twenty-five minutes after swallowing the tablets.

An hour after taking the mercury, his condition was as follows: A tall, muscular youth, weighing about 130 pounds, and apparently without anatomical defect, prostrated to the degree at which he could barely stand. Drops of cold perspiration running off forehead and face, and all clothing thoroughly soaked with perspiration. Skin and mucous membrane pale, features pinched, face has a slight cyanotic tinge. No corrosion about the mouth; mentally clear and manageable, vomiting every few minutes,—vomitus consisting of bloody water at first, later bile was present. Eyes deep set and encircled with dark rings; complains constantly of cramps in thighs and calves; has no abdominal pain. Slight pain at intervals in precordium; mouth temperature 97, respiration 24, pulse 90, very compressible and uniform in force, frequency and volume. Heart sounds distinct, no murmur or irregularity. Systolic pressure 110, diastolic 85. Abdomen flat, soft and painless. All extremities move freely; no cutaneous eruption anywhere. Very restless and exceedingly thirsty, though most of the water he drank would be immediately vomited. Urinalysis at this time showed albumin two-plus, casts three-plus, no sugar, no acetone, no pus, no crystals. Three hours later, a bloody diarrhea set in; there was no associated griping.

The patient was in the Infirmary eleven days. The cramps continued the first six days. Bloody vomit continued three days; bloody stools three and one-half days. Temperature remained above normal throughout his stay in the Infirmary,—highest reached, 101 the second day.

RECORD OF URINALYSIS

June 9: Spgr. 1009; Alb. 2-plus; casts 3-plus; acetone none; pus none; crystals 2-plus.
June 10: Alb. 3-plus; casts 3-plus; acetone none; pus none; crystals 2-plus.
June 11: Spgr. 1028; Alb. 4-plus; casts 3-plus; acetone trace; pus 1-plus; crystals 3-plus.
June 12: Spgr. 1019; Alb. 3-plus; casts 2-plus; acetone trace; pus 2-plus; crystals 4-plus.
June 13: Spgr. 1015; Alb. 3-plus; casts 2-plus; acetone none; pus 2-plus; crystals 4-plus.
June 14: Spgr. 1008; Alb. 2-plus; casts 2-plus; acetone 2-plus; pus 3-plus; crystals 4-plus.
June 15: Spgr. 1007; Alb. 1 plus; casts none; acetone 3-plus; pus 2-plus; crystals 4-plus.
June 16: Spgr. 1005; Alb. trace; casts none; acetone 3-plus; pus 1-plus; crystals 2-plus.
June 17: Spgr. 1011; Alb. none; casts none; acetone 2-plus; pus none; crystals 4-plus.
June 18: Spgr. 1015; Alb. none; casts none; acetone 2-plus; pus none; crystals 4-plus.
June 19: Spgr. 1018; Alb. none; casts none; acetone 1-plus; pus none; crystals 4-plus.
July 18: Spgr. 1018; Alb. none; casts none; acetone none; pus none; crystals none.
July 21: Spgr. 1020; Alb. none; casts none; acetone none; pus none; crystals none.

Only granular casts were found, no blood casts were ever found. Traces of blood by chemical test were discovered the second and third days.

Urinary Output.—Nine, three ounces; 10, fifteen ounces; 11, twenty-two ounces; 12, thirty-three ounces; 13, forty-three ounces; 14, sixty-nine ounces; 15, fifty ounces; 16, thirty-four ounces; 17, fifty-eight ounces; 18, forty-four ounces; 19, sixty ounces.

Treatment.—After the primary stomach washing, the stomach tube was laid aside and used no more. Throughout the attack he was advised to drink all the water possible,—the quantity retained varying from day to day. Every day he received an ounce of mineral oil, morning and night. He was given mucilage of acacia, three ounces, every four hours for five days, then three times daily for six days. Potassium acetate thirty grains in eight ounces

of water was given by enteroclysis every four hours for six days, after which renal function was encouraged by fifteen grains each of potassium acetate and citrate in a glass of water by mouth every four hours. An ampule of digifoline was given hypodermically every four hours for four days. On the 10, 11, and 12 Fischer's solution was given intravenously in the following quantities: 850 c.c.; 900 c.c.; 700 c.c. From the fourth day on Dobell's solution was used as a mouth wash. He took no food the first five days. Milk was given for the next five days, when soft egg and cereals were added. As you will see from the urinalysis, his kindneys cleared up rather promptly and examination today showed a perfectly normal urine.

July 21. The patient is back at work, looking well and feeling fine. He eats anything he wants and has no gastrointestinal disturbances resulting.

BIBLIOGRAPHY

[1]Fantus, B.: JOUR. LAB. AND CLIN. MED., 1916, i, 879.
[2]Fantus, B., and Hyatt: JOUR. LAB. AND CLIN. MED., 1917, ii, 815.
[3]Sabbatani, L.: Bioch. Centr., 1906, v, 502.
[4]Hall, G. W., Culbertson and Slaght: Jour. Am. Med. Assn., 1916, lxvi, 2062.
[5]Wilms, J. H.: JOUR. LAB. AND CLIN. MED., 1917, ii, 445.
[6]Haskell and Courtney: JOUR. LAB. AND CLIN. MED., 1917, iii, 110.
[7]Burmester, W. H.: JOUR. LAB. AND CLIN. MED., 1917, ii, 500.
[8]MacNider, Wm. de B.: Jour. Exp. Med., 1916, xxiii, 171; 1917, xxvi, 19.
[9]Lewis, D. S., and Rivers: Johns Hopkins Hosp. Bull., 1916, xxvii, 193.
[10]Sansum, W. D.: Jour. Am. Med. Assn., 1918, lxx, 824.
[11]Barbour, H. G.: Jour. Am. Med. Assn., 1915, lxiv, 736.
[12]Burmester and McNally: Jour. Med. Research, 1917, xxxvi, 87.
[13]Haskell, Carter and Coffindaffer: Jour. Am. Med. Assn., 1923, lxxxi, 6, 448.
[14]Lambert and Patterson: Arch. Int. Med., 1915, xvi, 865.
[15]Sollman: Manual of Pharmacology, Ed. 2, 1922, p. 922.

Rockefeller Institute of Medical Research

The Rockefeller Institute for Medical Research has announced the release of the drug known as Tryparsamide for use in the treatment of human and animal trypanosomiasis (African sleeping sickness and *mal de caderas*) and selected cases of syphilis of the central nervous system. This action is based on results reported from clinical investigations which have been in progress for several years. The drug will be manufactured by the Powers-Weightman-Rosengarten Co. of Philadelphia, and will become available through the regular trade channels about January 1, 1925. In releasing the drug for the benefit of the public, the Rockefeller Institute desires it to be known that the Institute does not share in any way in profits that may be derived from the sale of the drug and that, with the cordial cooperation of the manufacturers, provision has been made for the maintenance of a schedule of prices on as low a basis as possible.

THE EFFECT OF HEMOLYSIS ON THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF SERUM AND PLASMA*

By Chi Che Wang, Ph.D., and Augusta R. Felsher, B.S., Chicago, Ill.

IN articles dealing with the calcium and phosphorus content of blood it is often very difficult to ascertain whether the author used serum or plasma as a basis for his conclusions. Often, indeed, the two are used synonymously. In an article published in 1922, for instance, the heading of a table is given as follows: "Concentration of Na (or K, Ca and Mg) in Blood Serum and Corpuscles of Normal Adults," but the heading in the table reads "Plasma" instead of serum.[1] Many similar instances might be cited. We therefore undertook to determine whether the calcium and inorganic phosphorus content of serum and plasma were identical or varied, and what the trend of variation might be. Since blood for quantitative chemical estimations may show varying degrees of hemolysis, it is important to know also whether this factor has any bearing upon quantitative results. Further comparative determinations were, therefore, made on serum and plasma from hemolyzed and nonhemolyzed blood.

Experimental.—Two samples of blood of approximately 30 c.c. each were drawn from a sheep. One was prevented from clotting by the addition of sodium citrate in the proportion of 40 mg. to 10 c.c. of blood. Each sample was subdivided into two portions and one portion of each was hemolyzed by the addition of three drops of water. Calcium and inorganic phosphorus were determined on the centrifuged supernatant. For the determination of calcium, Kramer and Tisdall's[2] method was followed. The method of the same authors[3] was used for calcium in hemolyzed blood. For inorganic phosphorus Briggs'[4] modification of the Bell-Doisy phosphate method was employed. Determinations were made immediately after the blood was drawn, and the figures reported are the averages of duplicates. Two sheep furnished the blood used.

Discussion.—The table shows that there is no difference in the amount of inorganic phosphorus present in serum and in plasma. This is in agreement with the work reported by Tolstoi.[5] Hemolysis does not affect the phosphorus content of either the serum or the plasma. The calcium content, however, showed consistently somewhat lower figures for plasma than for serum. This was true in nineteen of twenty-three specimens examined, which gave values of 0.2 mg. to 3.4 mg. lower than the corresponding tests on serum. The four remaining cases showed a difference in serum and plasma calcium which was within experimental error. Hemolyzed blood invariably gave a

TABLE I

CALCIUM AND PHOSPHORUS CONTENT OF HEMOLYZED AND NONHEMOLYZED SERUM AND PLASMA*

	CALCIUM						PHOSPHORUS (INORGANIC)			
	NONHEMOLYZED		HEMOLYZED				NONHEMOLYZED		HEMOLYZED	
			UNTREATED		TREATED WITH AMMONIUM ACETATE					
NUMBER	SERUM	PLASMA	SERUM	PLASMA	SERUM	PLASMA	SERUM	PLASMA	SERUM	PLASMA
1	11.5	8.2		10.5	9.2					
2	11.0			9.9	8.2	9.7	6.2		6.1	5.9
3	12.3	11.7	15.2	10.9	13.1	10.6	6.3	6.5	6.4	6.7
4	11.6	12.3	13.7	11.4	9.7	9.9	4.3	3.9	4.0	4.0
5	12.8	11.2	17.6	11.0	12.5					
6	15.1	11.9	18.2	15.2	14.3	9.9	5.8	5.5	5.6	5.2
7	13.8	12.6	16.7	13.9	10.4	10.4	5.9	5.9	5.9	5.2
8	15.3	13.3	17.5	14.8	14.9	11.1	6.0	6.0	6.0	5.9
9	11.6	11.3	17.7	16.7	13.7	12.3	4.4	4.5	4.7	4.8
10	12.5	11.6	16.6	16.3	11.6		5.5	5.6	5.7	5.2
11	16.2	12.8	18.3	15.3	14.1	11.0	3.8	3.7	3.7	3.7
12	14.0	13.3			13.1	10.4	4.8	4.4	4.6	4.4
13	15.3	13.9			13.8	12.5	4.5		4.6	4.3
14	16.5	16.3			14.0	13.9	5.0		5.1	4.6
15	13.2	13.5			11.1	11.0	4.8		4.7	4.3
16	13.6	12.8			12.8	10.6	4.6		4.9	4.8
17	12.8	12.1			12.8	12.0	4.7		4.7	4.8
18	12.2	11.9			11.1	10.8	4.8		4.8	4.8
19	11.3	10.1			11.2	11.0	5.0		4.9	5.0
20	13.4	13.1			12.4	11.1	4.8		4.7	4.7
21	12.4	11.5			11.5	12.1	4.7		4.9	4.8
22	14.2	13.0	— —		12.8	11.6	5.0		4.8	5.0
23	12.8	13.0			11.8	10.9	4.7		4.8	4.8
24	12.7	13.0			11.1	11.6	4.9		4.9	4.9

*Reported as mg. per 100 c.c. of blood.

higher calcium content in serum than nonhemolyzed. Slight hemolysis does not necessarily affect the amount of plasma calcium; thus, we found that out of ten specimens, three gave a slightly lower figure and seven higher than the nonhemolyzed blood. The percentage increase of calcium in hemolyzed serum was from 14 per cent to 53 per cent over that of nonhemolyzed serum; the corresponding increases for plasma were 10 per cent to 48 per cent over the nonhemolyzed plasma. Strange to say, in only two cases did the calcium content of hemolyzed plasma exceed that of nonhemolyzed serum.

Kramer and Tisdall[3] stated that hemolyzed serum might be used for calcium determinations if treated with ammonium acetate. We accordingly treated both the hemolyzed serum and hemolyzed plasma by their method and determined the calcium content. Our results show lower calcium values in both serum and plasma when hemolyzed and treated with ammonium acetate than in the nonhemolyzed serum and plasma. Twenty-one out of twenty-four serum specimens showed values ranging from 0.9 per cent to 25 per cent lower than the nonhemolyzed serum. Of twenty specimens of plasma, seventeen gave values ranging from 0.8 per cent to 22 per cent lower than the nonhemolyzed plasma. The percentage variation is summarized in Table II. The difference is very likely due to the fact that in precipitating the iron from the hemolyzed serum or plasma, some of the calcium is likewise brought down. This difficulty might be overcome by reprecipita-

TABLE II

COMPARISON OF SERUM AND PLASMA CALCIUM IN HEMOLYZED AND NONHEMOLYZED BLOOD

NO.	INCREASE OF SERUM Ca OVER PLASMA Ca		INCREASE OF HEMOLYZED SERUM Ca OVER NONHEMOLYZED SERUM Ca		INCREASE OF HEMOLYZED PLASMA Ca OVER NONHEMOLYZED PLASMA Ca		INCREASE OF NONHEMOLYZED SERUM Ca OVER TREATED HEMOLYZED SERUM Ca		INCREASE OF NONHEMOLYZED PLASMA Ca OVER TREATED HEMOLYZED PLASMA Ca	
	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent
1	+3.3	+40.2			+2.3	+28.1	+2.3	+25.0		
2							+2.8	+34.2		
3	+0.6	+ 5.1	+2.9	+23.6	-0.8	- 6.8	-0.8	- 6.1	+1.1	+10.4
4	-0.7	- 5.7	+2.1	+18.1	-0.9	- 7.3	+1.9	+19.6	+2.4	+24.2
5	+1.6	+14.3	+4.8	+37.5	-0.2	- 1.8	+0.3	+ 2.4		
6	+3.2	+26.9	+3.1	+20.5	+3.3	+27.5	+0.8	+ 5.6	+2.0	+20.2
7	+1.2	+ 9.5	+2.9	+21.0	+1.3	+10.3	+3.4	+32.7	+2.2	+21.2
8	+2.0	+15.0	+2.2	+14.8	+1.5	+11.3	+0.4	+ 2.7	+2.2	+19.8
9	+0.3	+ 2.6	+6.1	+52.5	+5.4	+47.8	-2.1	-15.3	-1.0	- 8.1
10	+0.9	+ 7.8	+4.1	+32.8	+4.7	+40 5	+0.9	+ 7 8		
11	+3.4	+26.4	+2.1	+13.0	+2.5	+19 5	+2.1	+14 9	+1.8	+16.3
12	+0.7	+ 5.3					+0.9	+ 6 9	+2.9	+27.9
13	+1.4	+10.1					+1 5	+10.9	+1.4	+11.2
14	+0.2	+ 1.2					+2.5	+17.7	+2.4	+17.3
15	-0.2	- 1 2					+2.1	+18.8	+2.5	+22 7
16	+0.8	+ 5.9					+0.8	+ 6 3	+2.4	+20 8
17	+0.7	+ 5.8					0	0	+0.1	+ 0.8
18	+0.3	+ 2.5					+1.1	+ 9 9	+1.1	+10.2
19	+1.2	+11.9					+0.1	+ 0.9	-0 9	- 8.2
20	+0.9	+ 2.3					+1.0	+ 8 1	+2.0	+16 5
21	+0.9	+ 7.8					+0.9	+ 7.8	-0.6	- 5 0
22	+1.2	+ 9.3					+1.4	+10 9	+1 4	+12.1
23	-0.2	- 1.5					+1.0	+ 8 5	+2.1	+19.3
24	-0.3	- 2.3					+1.6	+14 4	+1 4	+12 1

tion, but it would involve much more manipulation and would increase the possibility of error.

Our findings show that it is not advisable to confuse the terms serum and plasma or to use them interchangeably when referring to calcium content, since there is some variation in the two cases. It is also well to keep clear the distinction between the hemolyzed and nonhemolyzed serum and plasma, as there is always a difference here in the calcium content. We would recommend that in reporting experiments on blood calcium, only nonhemolyzed blood be used, and serum or plasma specified.

SUMMARY

1. There is no difference in the phosphorus content of serum and plasma in either hemolyzed or nonhemolyzed blood.

2. The calcium content of nonhemolyzed serum is slightly higher than that of plasma. Hemolyzed blood, after being treated with ammonium acetate, gives a slightly lower calcium content than nonhemolyzed blood.

REFERENCES

[1]Kramer, B., and Tisdall, F. F.: The Distribution of Na, K, Ca. and Mg. between the Corpuscles and Serum of Human Blood, Jour. Biol. Chem., 1922, liii, 241.
[2]Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, Jour. Biol. Chem., 1921, xlvii, 475.

[3] Kramer, B., and Tisdall, F. F.: A Clinical Method for the Determination of Calcium and Magnesium in Small Amounts of Serum or Plasma. Bull. Johns Hopkins Hosp., 1921, xxxii, 44.

[4] Briggs, A. P.: A Modification of the Bell-Doisy Phosphate Method, Jour. Biol. Chem., 1922, liii, 13.

[5] Tolstoi, E.: The Inorganic Phosphorus of the Serum and Plasma of Ninety-one Normal Adults as Determined by the Bell and Doisy Method., Jour. Biol. Chem., 1923, lv, 157.

SPINOUS CELL CARCINOMA*

By L. A. Turley, M.D., Ph.D.,† Oklahoma City, Okla.

THE spinous cell carcinoma or, carcinoma developed from the stratum spinosum of stratified squamous epithelium has only recently been recognized as a distinct entity. Its position in the galaxy of tumors has not yet been generally accepted. This paper is presented to call your attention to some of the facts regarding this very common neoplasm, not only for what might be termed as an academic interest of exact identification and classification, but also for the practical reason that it is being recognized that tumors have definite habits and that it is as important to know the kind and origin of a tumor as it is to know that a given lesion is a tumor.

Ewing in his treatment of "Neoplastic Diseases" does not mention the spinous cell carcinoma as such, but includes this type of tumor in a heterogeneous group to which he gives the name of acanthoma. McCallum in his recent textbook on pathology includes it under an equally heterogeneous group of squamous cell carcinoma. The reason perhaps that this tumor has not been recognized, is, in the first place a general satisfaction on the part of clinicians and pathologists with the fact that a certain lesion is a carcinoma without any particular attention as to what class of tissues composes the tumor; and in the second place somewhat of a misapprehension has been perpetuated by histologists as to the manner of development and production of the various layers of stratified squamous epithelium, so that it has not occurred to the pupils of these histologists, who later became pathologists, that there was a possibility of a tumor developing from this particular layer.

Histologists have told us and still do tell us that in stratified squamous epithelium the act of multiplication takes place in the stratum germinativum, and that the cells so produced must of necessity be crowded outwards, and, in the process are modified, forming the stratum spinosum and finally become desiccated and keratinized to form the stratum corneum. Some few histologists however, have admitted that reproduction does take place in the very lowest layer of the stratum spinosum. A careful study of this type of epithelium reveals the fact that active reproductive processes are found as

far as the middle of this layer if not farther, and if we would add the facts of tumor formation as any indication of histologic processes, since in the so-called basal cell carcinoma we find little evidence of production of other types of cells of stratified squamous epithelium than those of the basal layer, we would be forced to conclude that the larger part of the stratum spinosum is produced by reproduction of the cells of this layer.

If we make a careful study of the factors which enter into the inciting of tissues to neoplastic growth and study the probability of the occurrence of the application of these facts to the various tissues, we would be led to the conclusion *a priori* that the stratum spinosum is the one from which we would expect the majority of tumors arising from stratified squamous epithelium to originate. Of such factors we would expect, first, to find tumors in tissues which normally preserved and exercised a rather high rate of reproductive processes, which is one of the reasons why epithelial and connective tissue tumors are the most common. Another fact would be that tumors arise from those tissues which are least stable, or in other words, have a rather weak or easily disturbed tissue set, which again would include the epithelium and connective tissues. Another fact would be the separation of living cells from an immediate supply of nourishment which would also include a supply of those internal secretions, which make for orderly development and reproduction. This fact coupled with the biologic law of an extra attempt at reproduction under the circumstances of unfavorable conditions for the carrying on of the vital functions would again affect most potently any tissue in a position similar to that of stratum spinosum. For here we have a tissue of living cells which must of necessity get their nourishment by means of percolation of nutritive fluids for some considerable distance from the blood vessels, and these same cells, being crowded farther and farther away from such meager supply, would not only suffer from reduced nutrition, but would be correspondingly freed from the controls to reproduction and its orderly progression, therefore readily grow wild or neoplastic.

If now we study the possibilities of tumor formation from stratified squamous epithelium, since there are two layers of living cells in this epithelium, we would expect to find at least three distinct types of tumors: one in which all three layers were represented in approximate equal proportions or the epidermoid carcinoma, one composed wholly or almost wholly of cells of the basal layer, or carcinoma basocellulare, and one in which the cells are all or for the greater part those arising from the stratum spinosum, or spinous cell carcinoma.

Two of these types have long been recognized. The third type has been but recently recognized by a few pathologists and the employment of this term is by no means general. Ewing's acanthoma includes the epidermoid carcinoma and the spinous cell carcinoma. The term of squamous cell carcinoma used by some pathologists is indefensible when applied to tumors arising from this epithelium for the reason that the only squamous cells in this type of epithelium are dead and hence cannot form tumors. If there is a squamous cell carcinoma at all it would be the endothelioma of Mallory, or in other

words the tumor arising from simple squamous epithelium. The mosaic appearance of the cells both in the stratum spinosum of stratified squamous epithelium and in the tumors arising from it is not confined to tumors arising from this tissue alone, but is characteristic of carcinomata arising from any epithelium. Likewise, the employment of the term, "squamous" applied to this form of epithelium by the histologists can only be defended on the grounds of failing to recognize the third dimension in their concept of tissues, forgetting that they are dealing with irregular polyhedrons rather than polygons. Such a mosaic picture can be obtained from a transverse section of cylindrical or cuboidal epithelium.

The spinous cell carcinoma is composed of fusiform polyhedral cells which in typical cases are approximately twice as long as broad. They have granular cytoplasm, and oval vesicular nuclei of comparatively good size. Although the cytoplasm is basophilic, or neutrophilic the nucleus is easily
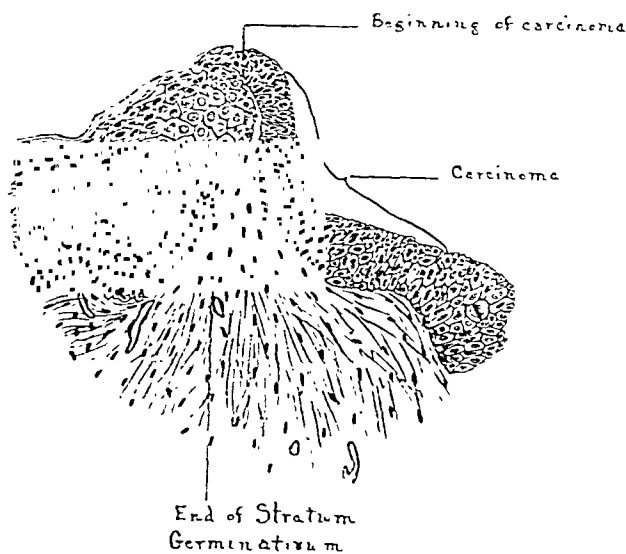
Drawing to explain Fig. 1.

made out. These cells tend to form sheets composed of many layers of cells. The longer axis of the cells of the middle layers of the sheets tends to be at right angles to the direction of growth. The longer axis of the border cells tends often to be parallel with the direction of growth. Sometimes, however, the sheet tendency is not apparent, the cells being arranged in great masses. and because of the destruction of all tissue up to and even including the walls of the blood and lymph vessels, these tumors are frequently diagnosed as sarcomata.

The typical features of this neoplasm, of course, are modified by the opportunity of the the tumor tissue to free and unhampered growth. If the tumor is invading a very dense tissue such as the corpus cavernosum the cells are often very small, densely packed together, with apparently very little or no cytoplasm, and might be easily mistaken for a small, dense, spindle cell sarcoma. We sometimes find in these carcinomata a tendency to pearl formation, but this is absent in rapidly growing tumors. Rarely are there any cells resembling those of the stratum germinativum.

There is often a more or less dense infiltration of lymphocytes, endothelial cells, connective tissue eosinophiles, and even polymorphonuclear leucocytes not only in the neighborhood, but within the tumor cell masses, which often confuse the picture. These tumors do not necrose as early or to such an extent as would other carcinomata in which the cell masses are as large. This is probably due to the fact that the spinous cell is one that is used to scant nutrition.

Fig. 1.—Showing origin of spinous cell carcinoma, (cervix uteri) x150. *A-B*, Normal surface of organ; *C*, hyperplastic epithelium, *D*, end of stratum germinativum; *E*, beginning of neoplasia; *F*, space left by necrosis.

The spinous cell carcinoma is rather slow in growth compared to cylindrical cell carcinoma. It destroys all tissues it penetrates and invades all tissues, yet it does not metastasize as readily as do most carcinomata. It has been known to destroy the cervix, upper vagina, part of the corpus uteri, and broad ligament, and invade the bladder and rectum without demonstrable metastasis. This late metastasis is probably due to the manner of growth which may be likened more to a liquid flowing over a surface rather than to a worm crawling along a burrow. That is, the spinous cell carcinoma crowds its way as a mass between the other tissues instead of penetrating

the tissues as slender cords of cells as most other carcinomata do, so that the detachment of individual cells or groups of cells from the masses is difficult, whereas the detachment of individual cells or groups of cells from slender cords is very easy.

These tumors sometimes appear as growths of short papilla above the surface, or as excavations in which the walls and floor are composed of stalactites. This appearance is due more to the necrosis of the invaded tissue between the convoluted sheets of tumor tissue than to a real papilla forma-
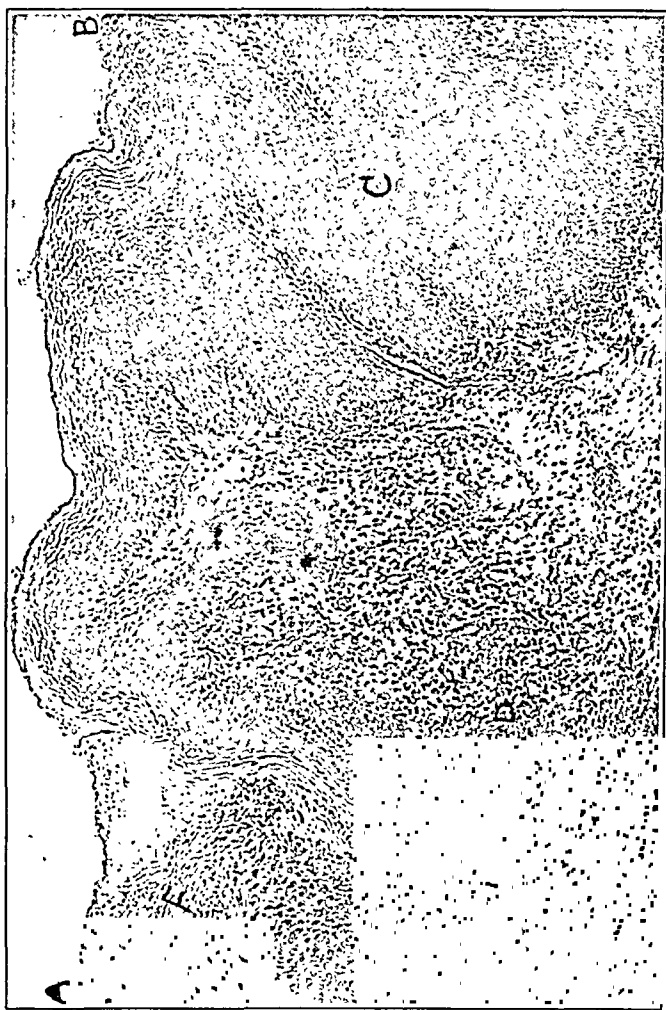
Fig. 2.—Showing origin of spinous cell carcinoma, burrowing type, x150. A-B, Surface of organ; C, hyperplastic epithelium; D, end of stratum germinativum; E, beginning of neoplasia; F, atrophied epithelium.

tion; however the true papilla formation is sometimes present. But occasionally, instead of appearing on the surface the tumor burrows deep into the tissues causing widespread destruction of other structures and leaving no trace or sign on the surface of its existence. It not infrequently happens that surgeons and gynecologists overlook this burrowing type, not suspecting the presence of the tumor because of the lack of disturbance of the surface. In my own experience as pathologist I have seen a number of such cases in which the cervix was amputated for other reasons, the presence of the carcinoma being revealed only on tissue examination.

The spinous cell carcinoma can and does occur on any part of the body where stratified squamous epithelium is found. It is most common, however, in those localities where the epithelium is kept moist, in other words on the interior, such as the mouth, esophagus, anus, vagina, and cervix uteri. In the latter site it is the most common tumor. In this location also it is found in its purest form.

The etiologic factors which seem most potent in the origin of these tumors are long-standing mild inflammation or trauma, or both. In the cervix it

Fig. 3.—Spinous cell carcinoma invading corpus cavernosum, x300. *A*, Connective tissue of organ. *B*, carcinoma.

is chronic cervicitis or endocervicitis alone, or plus erosion, or the previously mentioned inflammatory conditions involving an area of cervical repair where the epithelium has been turned in.

It is the province of this paper to deal almost wholly with the spinous cell carcinoma in its typical form. There are, of course, many modifications and combinations of this with other forms met, as is true of all tumors. But if those of you who do tissue work will bear in mind the points brought out, I am sure you will be surprised, if you have not already been so, at the frequency of the occurrence of this type of tumor, and also I am sure you will be convinced of its histogenesis. My own attention was first called to it in an accidental case where the origin of the tumor was very apparent.

AN ADEQUATE LABORATORY SERVICE FOR THE HOSPITAL*

By Philip Hillkowitz, B.S., M.D., Denver, Colo.

ONE of the most important requisites for a well-conducted institution for the care of the sick is the provision of proper laboratory facilities. From the very beginning of the movement for standardization of hospitals it was recognized that no exact scientific work in the diagnosis and treatment of disease could be carried out without the aid of the laboratory.

Paragraph 4 of the list of minimum standards of requirements enunciated by the American College of Surgeons reads: "That clinical laboratory facilities be available for the study, diagnosis and treatment of patients, these facilities to include at least chemical, bacteriologic, serologic, histologic, radiographic and fluoroscopic service in charge of trained technicians."

The College has wisely refrained from laying down definite or detailed specifications as to the kind or amount of clinical laboratory service to be given in the various classes of cases that are admitted to the hospital. It has left it to time and to the discretion of each hospital to work out its own problem in this particular.

A sufficient period has now elapsed since the inauguration of the standardization movement to advance a tentative program as to what constitutes adequate laboratory service. Daily conference with clinicians and surgeons and exchange of views between them and the pathologist during the past three years have greatly helped to clarify the question and to formulate certain basic principles which should invite universal approval.

We may first consider the so-called routine laboratory examinations, performed on every patient admitted to the hospital, regardless of the nature of the ailment. A clinical and microscopic examination of the urine comprising the determination of the specific gravity, reaction, the test for albumin and sugar and a search for casts, pus and crystals, is of course a *sine qua non* in every modern hospital. No patient, except in emergency cases, should go to the operating table or under the anesthetic without the accompanying report of the results of the urinalysis. It is as much part and parcel of hospital routine as obtaining the temperature and pulse, a procedure to which the patient is entitled by right as included in his implied contract with the hospital management. Next comes an examination of the blood. The extent of the investigation naturally varies with the case, and there is divergent opinion as to what determinations should be carried out routinely. On the one hand we must, in the interest of efficiency, avoid unnecessary and useless examinations which are time-consuming and constitute a great strain on the working personnel in the laboratory besides taking their energy away from

more essential duties. On the other hand, however, we cannot, in our zeal for economy of effort, overlook the possible accidental discovery of unlooked for important findings in more elaborate routine examinations. Between the Scylla and Charybdis we have to steer a middle course. It is, therefore, conceded that a white count of the blood is indicated in every case, this being substituted by a determination of the coagulation time in the ordinary tonsillectomy cases. Where the enumeration of the white blood corpuscles exceeds the normal a differential count should be made as a routine measure.

Another basic principle, of greatest importance to the surgeon, is the obligatory examination by a pathologist of all tissues removed at operations.

The examinations above specified of the urine, blood and tissues constitute what may be termed the basic routine laboratory examinations. These alone, however, would not fulfill in letter or spirit the requirements of adequate laboratory service. The enormous strides made in the diagnosis of disease by calling into requisition the recent contributions of chemistry, bacteriology, immunology and allied sciences make the laboratory an indispensable component of the hospital's armamentarium, to be placed at the disposal of the profession. Hence the equipment and personnel should be such as to enable the performance of the various laboratory tests employed in the modern practice of medicine when these are required by the attending physician. Accurate quantitative determination of urinary constituents, the examination of gastric contents and of feces and cerebrospinal fluid; the newer methods of blood chemistry, serologic procedures such as the Widal reaction and Wassermann test, blood cultures, preparation of vaccines and similar bacteriologic and clinical microscopic procedures ought to be carried out in the modern hospital laboratory.

Some of the more complicated tests, performed at infrequent intervals, requiring special apparatus, need not necessarily be done on the hospital premises, but may be performed by the pathologist in his private laboratory if his own facilities be better.

The examination of tissue removed at operation or for diagnosis is one of the most important functions of the hospital pathologist. There is no need to emphasize the value to the surgeon of the microscopic examination of histologic sections. Occasionally, a voice is heard in deprecation of the necessity of sectioning a manifestly gangrenous appendix and still louder are the protests against sectioning tonsils. The objections, be it mentioned parenthetically, are not against the scientific procedure *per se*, but against the cost of the examination added on the patient's already burdensome hospital bill. Of this, however, more anon. Leaving tonsils for the present out of consideration as a mooted question, we may lay it down as axiomatic that all tissues should be examined both macroscopically and microscopically. With the best of scrutiny and expert naked eye review of a specimen, some conditions will escape observation that would be revealed by the microscope. If tissues were examined only when requested by the surgeon, we should then be back in the pre-standardization era. Letting down the bars on certain kinds of tissue would quickly land us there. For this reason, we would make no exception in the case of tonsils

which are worthy of the same respect as the appendix or the ovary. And while utilitarian value to patient may not be immediately apparent in every case, both the physician and the patient are happier in the consciousness that all the scientific armor of the laboratory has been brought to bear on the case.

We are wont to speak of the laboratory somewhat metaphorically as an impersonal thing. Just as in referring to the operating room we desire principally to imply the surgeon, so in our use of the word "laboratory," we really designate the pathologist.

His rôle in the hospital is not merely to carry out or supervise the performance of the various routine or requested tests, but to interpret the results in terms of the patients. The clinician finds the pathologist willing to see or talk the case over with him, ready to suggest some laboratory procedure that would throw light on an obscure diagnosis. Together, by coordinating the clinical and laboratory findings, they are able to utilize their talents for the benefit of the patient. In short, the pathologist is a consultant in medicine. What is said here of the pathologist and the clinical laboratory applies with equal force to the roentgenologist and the x-ray laboratory. We, therefore, consider the wording of the minimum requirement for laboratories, "under the charge of trained technicians," rather unfortunate,* for no matter how highly trained they may be (and the training is anyhow mostly obtained from the pathologist) they have their limitations and should not be entrusted with such a grave responsibility as interpretation of laboratory determinations. To the stereotyped rejoinder that the clinician can do his own interpretation of clinical and x-ray laboratory findings, we may adduce the evidence that with the multiplicity of knowledge that has to be mastered nowadays by the physician only few can possess the happy faculty of keeping abreast of the advance in laboratory research.

It may be said in extenuation of the modest requirement of a trained technician rather than of the ideal, the trained pathologist, that the College was well aware of the deplorable lack in this country of competent physicians skilled in clinical pathology and the inability of hospitals to secure such service. As against the old evil of assigning the laboratory to some staff physician with whom it was a side issue or to an interne who was more interested in surgical technic, it is far better to have a trained technician in charge. Adequate laboratory service, however, means the conduct of the laboratory under the direction of a clinical pathologist who, according to the definition of the American Society of Clinical Pathologists, is a physician who has had at least two years' experience in chemistry, bacteriology, serology, clinical microscopy and tissue diagnosis. It is a specialty which requires a preparation, training, code of ethics and ideals similar to what the American College of Surgeons demands of those aspiring to be Fellows.

Clinical pathology, therefore, should not be, as intimated in some quarters, a stepping-stone to internal medicine or surgery, howsoever valuable a knowledge of this may be in the practice of these branches, but an end in

*The phrase has since been changed to read "under competent supervision" which is interpreted by the official bulletin of the American College of Surgeons as being "best done through the medium of a clinical pathologist."

itself, a career, a life's work. The technic of a test may be learned easily even by the layman. The proper evaluation of the findings requires a broad knowledge of medicine. The exact diagnosis of pathologic tissue beset by so many pitfalls, requires years of experience. Hence the futility of entrusting this serious task to one who is looking forward to abandon it for some other and more lucrative field of activity.

And this brings us to the painful consideration of the crux of the problem, the economic or financial side of the laboratory, which one loathes to inject into this scientific gathering but which, alas, cannot be avoided. In this prosaic world adequate service, laboratory or what not, cannot be had without proper compensation. The present famine in pathologists for whom hospitals in the country are clamoring is solely due to the inadequate reward offered for their talents. The knowledge acquired by the clinical pathologist in fitting himself for his specialty forms a wonderful asset in the domain of surgery or of internal medicine, both of which beckon to him and lure him on with promise of wealth and fame. Even morally his status is often confounded with that of a mere technician. It is not surprising therefore that men of promise in clinical pathology gravitate to other specialties.

The solution to this question resides in a recognition of the fact that laboratory service should not be considered as something furnished by the hospital like room, board, light or heat, but as a medical consultation indispensable to the patient and paid for by him in accordance with his means.

This is not the occasion to enter into details of financing the hospital laboratory; suffice it to say that in our opinion it should be self-supporting. The income should be derived from charging the patients for the services rendered on a reasonable and equitable basis.

In view of the scarcity of pathologists and the necessity of their adequate compensation, it is expedient for one or more hospitals to engage the services of a directing pathologist who will spend the requisite time daily in each institution to supervise the work of the technicians, interpret the findings and make out the tissue diagnosis.

In this way may be solved the problem of the small hospital in the rural communities which are at present without laboratory facilities or incompetently served by a technician. A pathologist from the metropolitan center may visit these institutions in outlying towns at periodical intervals much in the way of a circuit preacher. He will control the work of the technician and diagnose the tissue sections. In addition the pathologist will serve as a stimulus to the medical staff in maintaining the program of standardization in other particulars, such as record keeping and staff meetings. The pathologist in furnishing adequate laboratory service is in the best position to keep up the scientific spirit of the institution as planned by the requirements of the American College of Surgeons.

His work should not stop with merely doing what is asked of him by the clinician. It is also a part of his function to initiate scientific work by tactful suggestion to the physician in casual interviews or at the monthly staff meetings. It is at the monthly gathering of the hospital staff that the pathologist

is called on to defend his diagnoses, which sometimes run counter to those of the surgeon. On these occasions he can advance the cause of scientific medicine by calling attention to new laboratory procedures in diagnosis of disease or in demonstrating interesting pathologic specimens from the operative material of the hospital and from autopsies. Particularly should the pathologist emphasize the necessity of postmortem examination and solicit the attending physician to secure permission, for the percentage of autopsies obtained is an index of the scientific status of the institution.

Lastly we must not omit the proper keeping of records in the laboratory as an essential to adequate service. Not only should a duplicate of all reports be kept in proper books or card index but a classification of diagnoses made on tissue sections should be maintained for ready reference. One or two slides of each section should be carefully catalogued and preserved for future reference. A monthly report of the activities of the laboratory should be presented at the staff meetings.

In the manner above outlined, the laboratory personnel and the pathologist can best be of service to the clinician and exemplify the spirit of standardization of doing the greatest good to the patient.

THE OCCURRENCE OF PARATYPHOID AGGLUTININS IN SPECIMENS SUBMITTED FOR THE TYPHOID AGGLUTINATION TEST*

By Ruth Gilbert, M.D., and William M. Groesbeck, B.S.†

THE clinical course of typhoid fever is so variable that similarity of the symptoms to those of a surprisingly large number of other diseases may at times cause a confusion in diagnosis. At least two other members of the typhoid-colon group of bacilli, namely, paratyphoid bacilli A and B, are the etiologic factors in typhoid-like diseases, often considered clinically almost identical with typhoid fever. Hence, it has been the custom in this laboratory to make a routine examination for paratyphoid agglutinins on all specimens of blood submitted for the typhoid agglutination test.

In the six years, from 1918 to 1923, during which such examinations have been made, 13,759 specimens of blood have been received for the typhoid agglutination test. The results obtained upon one hundred and fifteen of these have been excluded from the present study, since only tests for typhoid agglutinins were made. Thus, the actual number upon which paratyphoid tests were made is 13,644.

Thirteen thousand and forty of these specimens were tested, as a routine procedure, in the 1:40 dilution of the blood with B. paratyphosus A and B and if a reaction was obtained the test was repeated in the three dilutions,

1:20, 1:40 and 1:80. The results, summarized in Table I, have been divided into those in which complete agglutination occurred and those in which a partial agglutination reaction was observed (i.e., partial in 1:40 dilution).

TABLE I

RESULTS OF ROUTINE TESTS FOR PARATYPHOID AGGLUTININS

YEAR	TOTAL SPECIMENS TESTED	COMPLETE AGGLUTINATION		PARTIAL AGGLUTINATION	
		NUMBER	PER CENT	NUMBER	PER CENT
1918	2794	0	–	0	–
1919	2228	1	0.05	3	0.13
1920	2143	6	0.28	44	2.05
1921	2495	3	0.12	24	0.96
1922	1838	0	–	2	0.11
1923	1542	2	0 13	18	1.16
Total	13040	12	0.09	91	0.7

An average of 0.09 per cent (approximately 1 in 1000) routine agglutination tests has shown complete agglutination of paratyphoid bacilli and 0.7 per cent (7 in 1000) has shown partial agglutination.

Six hundred and four specimens were tested for paratyphoid agglutinins at the request of the physician or because the histories indicated that paratyphoid fever was suspected. The results of these tests are given in Table II.

TABLE II

RESULTS OF TESTS FOR PARATYPHOID AGGLUTININS WHERE PARATYPHOID INFECTION WAS INDICATED

YEAR	NUMBER OF SPECIMENS TESTED	COMPLETE AGGLUTINATION		PARTIAL AGGLUTINATION	
		NUMBER	PER CENT	NUMBER	PER CENT
1918	102	5	4.9	4	3.9
1919	85	3	3 5	0	–
1920	96	0	–	9	9.4
1921	134	2	1.5	8	6.0
1922	99	2	2.0	3	3.0
1923	88	0	–	8	9.0
Total	604	12	2.0	32	5.3

An average of 2.0 per cent (20 in 1000) of these Tests has shown complete agglutination and an average of 5.3 per cent (53 in 1000) has shown partial agglutination.

Conclusion.—The infrequency with which the presence of paratyphoid agglutinins has been demonstrated indicates that the test for them may be omitted from the routine examination of specimens submitted for the typhoid agglutination test without materially detracting from the value of these examinations. Whenever paratyphoid infection is indicated, however, the test for the presence of these agglutinins should be made.

THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS

IV. The Diuretic Effect of Benzyl Alcohol, Benzyl-Acetate and Benzyl-Benzoate

By Charles M. Gruber, Ph.D., M.D., St. Louis, Mo.*

MACHT[1] studied the toxicity of the benzyl esters, also their effect upon kidney function on dogs. He found that prolonged administration of benzyl-benzoate and benzyl-acetate, both by mouth and by injection produced no chronic intoxication. Examination of the urine indicated no impairment of the kidneys, and functional tests with phenolsulphonephthalein showed no change in kidney function.

While testing the effect of oral administration of the benzyl esters upon the alimentary canal in dogs[2] it was noted that there was frequent urination in addition to frequent vomiting and defecation. At first this action was thought to be due to the relaxation of the bladder sphincter and the copious drinking of water. However, in a later series of experiments[3] with the intravenous administration of benzyl alcohol, benzyl-acetate and benzyl-benzoate these substances were found to produce circulatory changes within the kidneys. These facts suggested that it would be interesting to note if the above substances had an effect on the secretion of urine.

METHOD

Dogs and rabbits were employed. The former animals were anesthetized either with paraldehyde 1.8 ml. per kg. by mouth or with ether by inhalation. Urethane, 2 gm. per kg. body weight, by stomach or ether by inhalation, were used for the latter animals. The rabbits were fed generous quantities of green cabbage several days before the experiments were performed.

The blood pressure, when recorded, was always taken from the left carotid artery with a mercury manometer. A 2 per cent sodium citrate solution was employed as an anticoagulant. A chronometer indicating either 5, 15 or 30 second intervals, was employed. When the blood pressure was recorded the chronometer also indicated the zero blood pressure level.

Cannulas were placed in each ureter and the drops of urine secreted were recorded on the kymograph by receiving and recording tambours.

Benzyl alcohol, benzyl-acetate and benzyl-benzoate were used in 10 per cent emulsions made with 5 per cent acacia in Ringer's solution.[3] These were injected slowly into the lateral ear vein of the rabbit. The pure preparations were used for intramuscular and intraperitoneal injections. In some experiments the intestine was ligated near the pyloric sphincter.

RESULTS

Intravenous Administration.—The results upon intravenous administration were not satisfactory. In many instances the rate of urine flow was markedly diminished and in some cases there was complete suppression. The results were influenced by the quantity and rate of injection of the drug. The suppression appeared to be secondary to the effect of the drug upon the heart muscle, respiratory system and blood pressure. See Gruber.[3] The greater the fall in blood pressure the greater the suppression of urine. In some instances intravenous injections were followed by marked augmentation in the rate of urine flow. Fig. 1, which was obtained from a 3 kg. rabbit, illustrates the normal flow of urine taken as control. At X^1 2.5 ml. 5 per
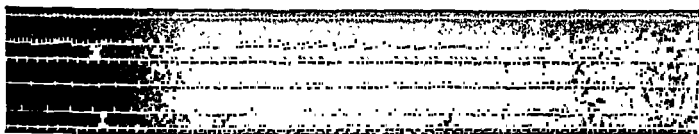
Fig. 1.—Rabbit 3 kg. Urethane anesthesia. Cannulas in both ureters. Upper record left kidney, middle record right kidney secretions in drops. I. Control rate of urine flow in drops. At X^1 2.5 ml. acacia solution five per cent and at X^2 2.5 ml. acacia solution five per cent containing ten per cent benzyl-benzoate were injected intravenously; II. Continuation of record I. Time in thirty seconds. This and all following illustrations are reduced ⅓ or more.
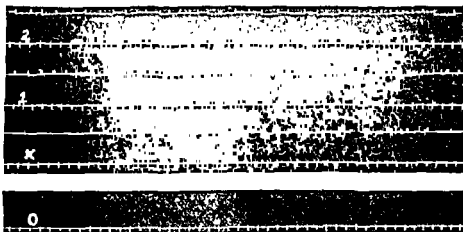

Fig. 2.—Rabbit, urethane anesthesia. Cannula placed in left ureter near the hilus of the kidney. O, control rate of urine flow for thirty-seven minutes. X, 1 ml. pure benzyl alcohol was injected intramuscularly; 1, fifty minutes later; 2, one hundred minutes after X. Time in thirty second intervals.

cent acacia was injected into the lateral ear vein. There was produced no noticeable change in urine flow. At X^2 the same amount and concentration of acacia but containing 10 per cent benzyl-benzoate was injected intravenously. Within six minutes the urine flow was augmented. It was increased from about 1 drop per minute to about 8 drops per minute for each kidney. This augmented urine flow continued for ninety minutes longer than shown in the figure.

Intramuscular Administration.—That diuresis can be produced by these drugs when they are injected intramuscularly can be seen in Fig. 2. In this animal the cannula was placed in the left ureter near the hilus of the kidney. The normal rate of urine flow was recorded for thirty-seven minutes, of

which curve 0 is an example, before the injection was made. At X, 1 ml. pure benzyl alcohol was injected intramuscularly; curves 1 and 2 were taken respectively fifty and one hundred minutes later. In this animal the effect of the drug was shown in eight minutes. The maximum rate of urine flow was 2 drops per minute, whereas before the injection the rate was 1 drop per three minutes.

Intraperitoneal Administration.—The most satisfactory results were obtained by the administration of pure preparations of benzyl alcohol, benzyl-acetate and benzyl-benzoate intraperitoneally. The degree of increased urine flow and the duration of the latent period varied with the different animals used. In nearly all instances the duration of the latent period was shortest for benzyl alcohol but longest for benzyl-benzoate. This will be seen upon comparing Tables I, II and III. The average time of the latent period for benzyl alcohol in 22 animals thus studied was found to be about seven minutes, for benzyl-acetate in 10 animals eight minutes, and for benzyl-benzoate in 7 animals nineteen minutes.

In a previous communication[4] the author found no evidence to support Nielsen and Higgins'[5] claim that the different benzyl esters varied in their rate of action upon the blood pressure. They maintain that there was a difference and that this difference in rate of action was dependent upon the

TABLE I

TABLE SHOWING THE APPROXIMATE TIME INTERVAL BETWEEN THE TIME OF INJECTION AND THE BEGINNING OF AUGMENTATION IN URINE FLOW AFTER BENZYL ALCOHOL WAS INJECTED INTRAPERITONEALLY. MAXIMAL RATE OF FLOW OF URINE IN DROPS SHOWN IN TABLE

ANIMAL	WEIGHT IN KILOGRAMS	PURE BENZYL ALCOHOL IN ML.	TIME OF LATENT PERIOD IN MINUTES	MAXIMAL RATE OF URINE FLOW IN DROPS PER MINUTE			
				RIGHT KIDNEY		LEFT KIDNEY	
				NORMAL	AFTER BENZYL ALCOHOL	NORMAL	AFTER BENZYL ALCOHOL
Rabbit	2.0	1.0	10	...	...	0.5	2.5
	3.0	1.5	12	0.3	4.3	0.3	3.0
	3.2	1.6	7	0.7	5.3	...	...
	3.0	1.0	3	0.3	3.0	0.2	3.0
	5.0	2.5	3.5	3.0	5.3	2.5	5.5
	4.3	2.2	3	...	...	1.0	4.0
	3.9	2.0	3	1.0	4.0	...	...
	3.6	2.0	5	2.0	4.0	...	...
	3.7	2.0	14	0.3	1.7	...	...
	3.4	2.0	10	1.0	2.0	1.0	1.7
	3.9	2.0	4	2.7	5.0	4.7	7.3
	2.5	1.5	6	...	...	0.3	2.8
	2.5	1.0	3	4.0	6.5	4.0	6.0
	3.2	1.0	2	1.0	3.0	0.5	1.5
	3.0	1.0	20	0.5	1.5	0.3	0.8
	3.0	1.0	16	1.0	1.3	1.2	1.8
	2.3	1.0	1	1.5	6.5	1.2	6.5
	4.3	1.0	14	1.8	6.0	2.5	5.5
Dog	18.0	3.0	5	2.8	3.8	3.5	4.5
	14.5	5.0	7	1.8	2.0	..	...
	9.5	2.5	6	0.5	8.5	1.0	8.5
	14.1	1.0	2	2.0	4.0	2.0	5.5
Average			7.1	1.5	4.1	1.55	4.1
Increase in per cent					173		170

respective rates of hydrolysis. The above findings support Nielsen and Higgins' view that these esters may be converted into their components before producing their action. In this connection it must also be remembered that the rate of absorption from the peritoneal cavity will vary with their respective solubilities. Inasmuch as benzyl alcohol and benzyl-acetate are fairly soluble and benzyl-benzoate practically insoluble in water, one would naturally expect some difference in their rates of action.

The average increased rate of urine flow is slightly greater for benzyl-benzoate than for either benzyl-acetate or benzyl alcohol, it being about 215, 182 and 171 per cent respectively. The average increase was 190 per cent for the entire series of experiments.

Benzyl Alcohol.—Benzyl alcohol has a decided action upon the kidney function. See Figs. 3, 4 and 5 and Table I. Figs. 3 and 4 were obtained from experiments upon rabbits. It will be seen that in Fig. 3 the blood pressure

TABLE II

TABLE SHOWING THE APPROXIMATE TIME INTERVAL BETWEEN THE TIME OF INJECTION AND THE BEGINNING OF INCREASED URINE FLOW AFTER BENZYL-ACETATE WAS INJECTED INTRAPERITONEALLY. MAXIMAL RATE OF FLOW OF URINE IN DROPS SHOWN IN TABLE.

ANIMAL	WEIGHT IN KILOGRAMS	PURE BENZYL-ACETATE IN ML.	TIME OF LATENT PERIOD IN MINUTES	MAXIMAL RATE OF URINE FLOW IN DROPS PER MINUTE			
				RIGHT KIDNEY		LEFT KIDNEY	
				NORMAL	BENZYL-ACETATE	NORMAL	BENZYL-ACETATE
Rabbit	2.5	1.0	14.0	1.0	2 4	1.0	2.4
	3.0	1.3	7.5	2.0	4.0	...	...
	3.9	2.0	5 0	1.4	3.4	1.4	3.7
	3.4	2.5	10.0	0.3	1.3	...	...
	2.3	1.0	4.0	1 0	3.0	1.0	5.8
	2.3	1 0	6.0	0.5	3 0	0.5	3.0
	3.8	2 0	4.0	0.5	6.0	0.3	6.0
Dog	11.0	5 0	15.0	2.5	3.0	1.3	1.5
	15.0	5 0	10 0	2.5	3.0	2.0	2.5
	11.0	4.0	8.0	0.3	0.8	0.3	0.8
Average			8.3	1.2	3 0	1.0	3.2
Increase in per cent					150		220

TABLE III

TABLE SHOWING THE APPROXIMATE TIME INTERVAL BETWEEN THE TIME OF INJECTION OF BENZYL-BENZOATE INTRAPERITONEALLY AND THE BEGINNING OF INCREASED URINE FLOW. MAXIMAL FLOW OF URINE IN DROPS SHOWN IN TABLE

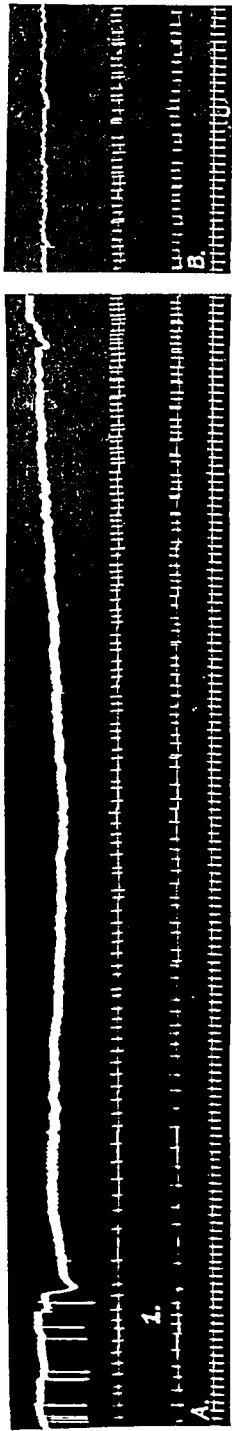| ANIMAL	WEIGHT IN KILOGRAMS	PURE BENZYL-BENZOATE IN ML.	TIME OF LATENT PERIOD IN MINUTES	MAXIMAL RATE OF URINE FLOW IN DROPS PER MINUTE			
				RIGHT KIDNEY		LEFT KIDNEY	
				NORMAL	BENZYL-BENZOATE	NORMAL	BENZYL-BENZOATE
Rabbit	2.1	0.5	25	0 5	3.0	0.5	2.0
	2.4	1.2	18	3.0	21.0	2.0	9.0
	3.0	1.5	45	1.0	5.0	1.0	6.0
	5.25	1.0	8	2.5	6.0	1.5	5.3
	3.0	1.0	4	8.0	12.5	5.8	8.3
Dog	10.3	5.0	23	1.5	3.5	1.0	2.5
	14.5	5.0	10	1.3	0 5	1.0	6.5
Average			19	2.5	8.2	1.9	5.7
Increase in per cent					228		200

Fig. 3.—Rabbit 4.3 kg. Urethane anesthesia. Top record is blood pressure, bottom record is time in 15 second intervals and zero blood pressure. Of the remaining records the upper is the rate of secretion of urine in drops from the right kidney and the lower from the left kidney. In this and the remaining records cannulas were placed in the ureters. A, 1. Intraperitoneal injection of 1 ml. pure benzyl alcohol. B, Record taken thirty minutes after terminus of A.
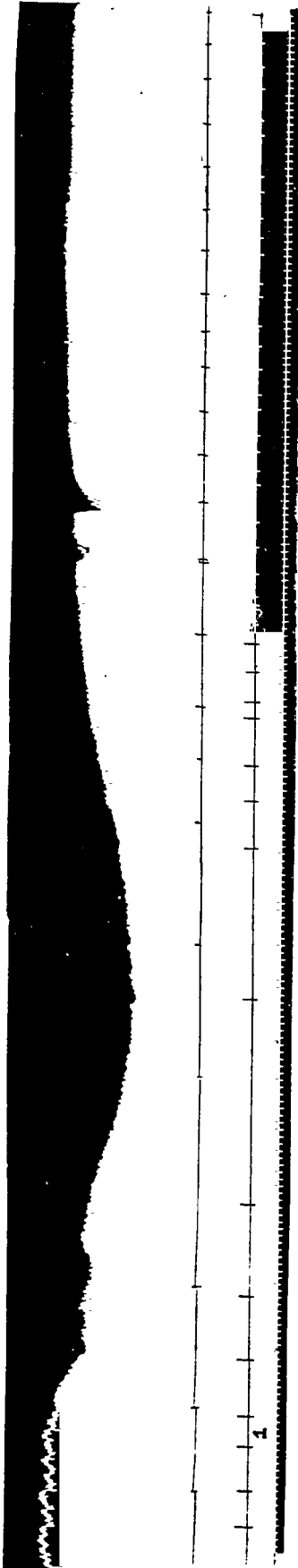
Fig. 4.—Rabbit 3 kg. Urethane anesthesia. Top record is the blood pressure, the bottom one is the time in fifteen second intervals and zero blood pressure. Of the remaining records the upper is the rate of secretion of urine in drops from the left kidney and the lower from the right kidney. 1. Intraperitoneal injection of 1 ml. pure benzyl alcohol.
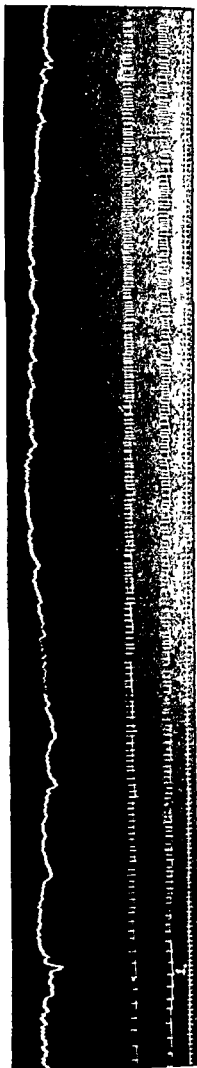
Fig. 5.—Rabbit, 2.3 kg. Urethane anesthesia. Same as Fig 4. Record continued for forty-five minutes at which time the blood pressure dropped to 84 mm. of mercury and the secretion of urine was 1.5 drops for the left and 2 drops for the right kidney per minute.
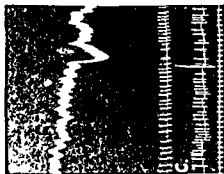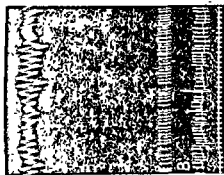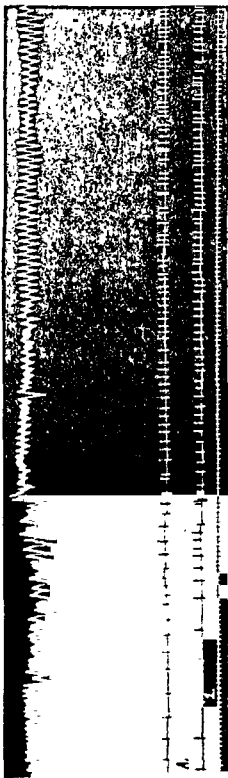

Fig 6.—Dog, 9.6 kg. Paraldehyde anesthesia. Same as Fig. 4 except as follows: A, 1, 2.5 ml. pure benzyl alcohol were injected intraperitoneally; B, fifteen minutes after end of A; C, thirty minutes after end of B.

fell after the intraperitoneal injection of 1 ml. pure benzyl alcohol. This sudden fall in pressure is accompanied by a slowing in the secretion of urine. Following this there is a more gradual fall in blood pressure, then a return to normal with a marked increase in the secretion of urine. In some instances, as illustrated in Fig. 4, the blood pressure fell to such an extent that the secretion of urine was almost stopped. In this instance, however, the blood pressure returned to normal and the urine secretion went above normal. In some other animals the blood pressure remained low during the remaining length of the experiment and anuria resulted.

In Fig. 5 the blood pressure did not fall after the injection of 1 ml. pure benzyl alcohol. It increased from 122 to 135 mm., which later returned to 116 mm. of mercury. Simultaneously with this change, the number of drops of urine excreted increased from 1.5 drops per minute to 6 drops for the left kidney and to 7 drops for the right kidney. That benzyl alcohol and its esters when injected intravenously can increase the blood pressure was shown by the author in a previous communication.[3] These results upon intraperitoneal administration further support the finding given there as do the observations obtained on the dogs used in securing Figs. 6 and 9.

Intraperitoneal injection of 2.5 ml. of pure benzyl alcohol was made in a dog weighing 9.6 kg. in Fig. 6. The blood pressure temporarily fell from 160 to 150 mm. of mercury but ten minutes after the injection it had risen to 170 mm. of mercury, falling gradually to 160 mm. during the next thirty minutes. Thirty minutes later, or seventy minutes after the injection, the blood pressure was only 110 mm. of mercury. As seen in B in Fig. 6 the secretion of urine increased rather rapidly, reaching a maximum forty-five minutes after the injection. Although the blood pressure dropped more than 50 mm. in C, nevertheless the rate of urine flow is very much faster than the control rate in A.

Benzyl-acetate.—Benzyl-acetate like benzyl alcohol has a short latent period. This is seen in Table II and Fig. 7. After the injection of benzyl-acetate in Fig. 7 the blood pressure gradually fell from 120 to 76 mm. of mercury. The excretion of urine rapidly increased and continued above the normal rate for more than two hours. Like benzyl alcohol, benzyl-acetate occasionally produced a rapid fall in blood pressure accompanied by anuria, from which the animals did not recover during the time of the experiment.

Benzyl-benzoate.—Figs. 8 and 9 are presented to show the effects of benzyl-benzoate upon the rabbit and dog respectively. In Fig. 8, curve 1 shows the normal rate of secretion of urine by both kidneys; 2 the injection of 1.2 ml. pure benzyl-benzoate intraperitoneally; and 3 and 4 the rate of secretion of urine fifty and one hundred minutes respectively after the injection. The rate of secretion before the injection was about 1 drop per minute which reached a maximum rate of 3 drops per minute after the injection. In this animal the blood pressure was not recorded.

The dog used in Fig. 9 weighed 14.5 kg. and received 5 ml. pure benzyl-benzoate intraperitoneally at 1. The blood pressure rose as the record shows from 120 to 142 mm. of mercury but later fell to 120 mm., curve C. After
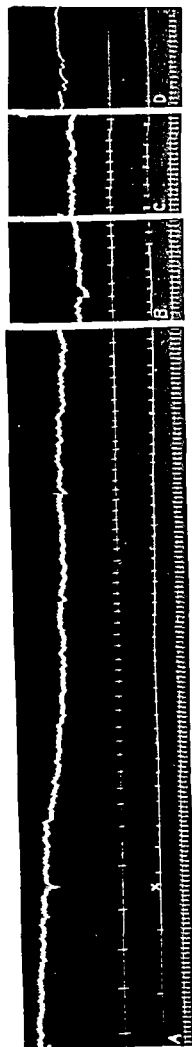
Fig. 7.—Rabbit, 3.8 kg. Urethane anesthesia. Same as Fig. 3, except as follows: X, 2 ml. pure benzyl-acetate were injected intraperitoneally. B, thirty minutes after end of A, C, one hour after B; and D, thirty minutes after C.
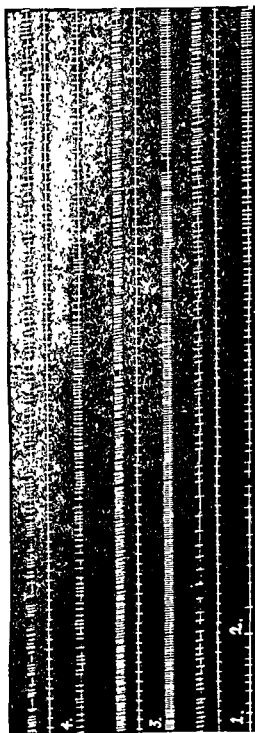

Fig. 8.—Rabbit 2.4 kg. Urethane anesthesia. Urethane given one hour before before operation. Top record secretion of urine in drops by right kidney, middle line in thirty second intervals, bottom record secretion of urine in drops by left kidney. 1. Control rate. 2. Intraperitoneal injection of 1.2 ml. pure benzyl-benzoate. 3 and 1. Records taken fifty and one hundred minutes respectively after the injection.
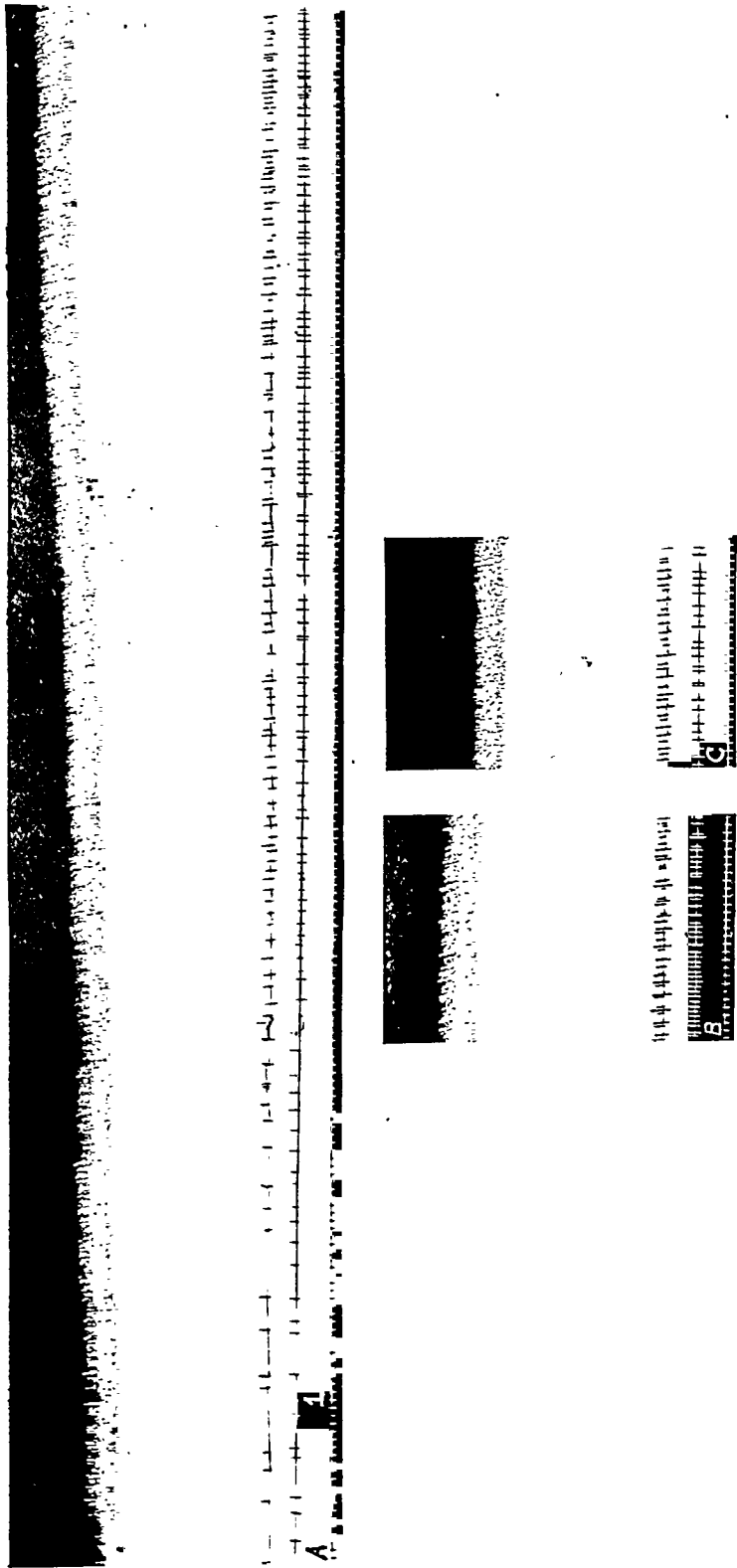
Fig. 9.—Dog, 14.5 kg. Paraldehyde anesthesia. Same as Fig. 6, except as follows; at 1, 5 ml. of pure benzyl-benzoate were injected intraperitoneally; B, fifteen minutes after A; C, twenty-five minutes after B.

the injection the rate of urine flow gradually increased from 1 and 1.3 drops to 6 and 6.3 drops for the left and right kidneys respectively.

DISCUSSION

From the above results it will be seen that benzyl alcohol and its esters benzyl-acetate and benzyl-benzoate increase the rate of urine secretion. Inasmuch as diuresis occurred even after pyloric sphincter ligation it cannot be due to relaxation of these smooth muscles thus permitting rapid emptying of the fluid contents of the stomach into the intestine. In a previous communication[3] plethysmographic records were shown demonstrating a decreased volume of the kidney in all the animals tested. In some instances the blood pressure was not changed nevertheless the kidney decreased in volume while at the same time the intestine increased in volume[3] (Fig. 9). Perfusion experiments confirmed Macht's[1] finding however, that the kidney vessels may dilate under the influence of benzyl alcohol and its esters.[3a] Whether benzyl alcohol and its esters irritate the kidney cells to further secretion, as is thought to be the case with calomel[6]; change the glomerular membrane so that fluids are filtered more readily, as some authors think to be the case with caffein[7]; decrease the rate of reabsorption of fluids in the tubules, as is thought to be the case with sodium sulphate[8]; increase the number of glomeruli secreting as has been found to be the case with caffein by Richards and Schmidt[9]; or increase intrarenal arterial blood pressure and increase volume flow through the kidney, cannot be stated at this time. Experiments are now being performed in an effort to determine if possible the mode of action of benzyl alcohol and its esters in producing this increased urine secretion. Our findings on the action of these drugs upon urine secretion support those of Macht's,[10] upon the circulation to the effect that rabbits are much more susceptible to the benzyl esters than are dogs. We found rabbits to respond by a much greater increase in the rate of urine flow than the dogs used in the experiments.

SUMMARY

1. Benzyl alcohol and its esters benzyl-acetate and benzyl-benzoate cause increased urine secretion when given either intravenously, intramuscularly or intraperitoneally.

2. The latent period between the intraperitoneal injection and beginning of increased urine secretion in 22 animals was found to be seven minutes with benzyl alcohol; eight minutes in 10 animals with benzyl-acetate and nineteen minutes in 7 animals with benzyl-benzoate.

3 Benzyl alcohol was found to increase the rate of urine flow 171 per cent in 22 animals, benzyl-acetate 182 per cent in 10 animals and benzyl-benzoate 215 per cent in 7 animals, an average of 190 per cent for the entire series.

4. The point of action of the drug has not been determined.

REFERENCES

[1]Macht: Jour. Pharm. Exper. Therap., 1918, xi, 433.
[2]Gruber: JOUR. LAB. AND CLIN. MED., 1923, ix, 30.

[3]Gruber: Ibid., 1923, ix, 92.
[3a]Gruber: Ibid., Fig. 10 C.
[4]Gruber: loc. cit., p. 109.
[5]Nielsen and Higgins: JOUR. LAB. AND CLIN. MED., 1921, vi, 388; ibid., vii, 69.
[6]Cushny: The Secretion of the Urine, Longmans, Green and Company, 1917, p. 184.
[7]Cushny: loc. cit., p. 180.
[8]Cushny: loc. cit., p. 146.
[9]Richards and Schmidt: Amer. Jour. Physiol., 1922, lix, 489.
[10]Macht: loc. cit., p. 427.

THE PREPARATION OF A SATISFACTORY POIKILOTHERMOUS ANIMAL*

By Robert W. Keeton, M.S., M.D., Chicago, Ill.

IN the course of an investigation into heat regulation it became necessary to prepare poikilothermous animals. This loss of the power to maintain a constant body temperature in the face of changing environmental temperatures results when the cervical cord of an animal is transected. Many of the investigators (see ref. 1-6) who have prepared such animals have subjected them to experimental studies within a short time (3 to 4 hours) of their recovery from the anesthetic. However, my experiments required that the animals recover from the immediate effects of the operation and return to their normal feeding routine. Freund and Strassman[7] have reported the most complete experiments on poikilothermous animals surviving for a number of days. Many of their rabbits lived from seven to ten days. They record, however, a rather high mortality occurring within the first twenty-four hours postoperative. These deaths were attributed to surgical shock, but no attempt was made to analyze further the factors involved. Since this type of animal is useful in physiology for teaching and demonstration purposes and offers an opportunity for the study of the vasomotor mechanism, it seemed advisable to present a technic which has proved successful in its preparation.

Selection of Animal.—In the preparation of a cervical cord animal, the rabbit offers some advantages over the dog. The phrenic nerve in the rabbit[8] arises chiefly from the fourth segment with small branches from the third and fifth. In the dog[9] it arises from the fifth, sixth and seventh cervical nerves. It is evident that the chances of damaging the phrenic nuclei are much greater in the dog than the rabbit. After section of the cord the motility of the intestines is increased[10] so that a diarrheic state readily establishes itself. With the rabbit this may be controlled by the character of the food. It never becomes troublesome. In the dog because of the frequent respiratory tract infections (sniffles, distemper) and this increased intestinal motility, the stools rapidly become watery, foul smelling, and streaked with blood. The use of bone ash in the food may help but it does not eliminate entirely

this bowel disturbance. Bed sores never develop in the rabbit. They always occur in dogs with high section if the animals live long enough. It is however quite difficult to maintain the body weight of the rabbits. They are helpless and as a result have to be fed with the hand. They eat slowly so that almost constant attention must be given to their feeding over a period of two to three hours daily, if anything like an adequate quantity of food is eaten.

Preparation of the Operative Field.—Rabbits readily develop staphylococcus infections. Such infections have been observed occurring spontaneously in the mammary glands of two otherwise healthy animals and in the axillary space of a third. If tincture of iodine be used for the sterilization of the field of operation, a slough will appear in a few days along the wound edges. The organisms invade this slough and if the animals be sacrificed at varying intervals following the operation the progress of this infection may be traced from the surface to the spinal cord. The application of 70 per cent to 95 per cent alcohol to the skin gives satisfactory antisepsis. It does not cause a slough and its use eliminates this type of wound infection.

Anesthetics and Heat Loss.—The operation can be performed under ether but the hazard is great. Rabbits die so readily under a simple ether anesthetic that it requires a definite period of training before an assistant can be regarded as a safe anesthetist. Then the ether predisposes to pneumonia an animal which at best is very susceptible to this form of infection. However, the important objection to ether lies in the very rapid loss of heat after section of the cord. Moore[11] has observed that the administration of ether to a normal rabbit for an hour may lower the rectal temperature 1.5° C. Our experience shows that within a short period following the transection of the cord a lowering of the temperature of 1° to 4° C. occurs. This fall was observed by the earlier investigators and was the subject of extended study. More recently their observations have been reviewed and summarized by Schönborn.[4] In my experience this fall occurs despite the protection of the animal by blankets and the application of external heat. Five to six hours are required to return the animal to a temperature of 38° C. and the rabbit normal (38 8°-39.4°) may not be reached for twelve hours.

If a local anesthetic (2 per cent novocaine) be used, the temperature may rise immediately after the section. In one case a temperature of 41° was noted and in two others a smaller elevation occurred. This recalls to mind the clinical experience that lesions of the cervical cord are often associated with hyperpyrexia. However, such rises are transitory and if the animal be not protected from heat loss the temperature will fall progressively. In the majority of cases there is a drop of 1° to 1.5° C. within twenty minutes of the section. If external heat be applied the temperature remains stationary or rises rapidly to normal. This is in sharp contrast to the prolonged lag present in the ether animals before the depression can be removed. The ether certainly exaggerates those changes in the heat regulating mechanism induced by cervical section.

Establishment of Reflex Block.—In four consecutive animals operated under deep surgical anesthesia, respirations ceased simultaneously with the section of the cord. In other animals the cord was cut under light ether anesthesia. They also stopped breathing with the completion of the section. This cessation of the respiration may have been due to a primary reflex respiratory inhibition or to a secondary one due to some form of acute circulatory failure. The injection of morphine 1.65 mg. and atropine 0.22 mg. in rabbits weighing 2 to 2.5 kilos eliminated this reflex factor when ether was employed.

In the use of novocaine, it was customary to give two injections of morphine (1.65 mg.) and atropine (0.22 mg.) at an interval of one hour. An even larger dose may be used with safety. After the exposure of the cord, a novocaine block was established. Not over 0.3 to 0.4 c.c. of 2 per cent novocaine should be injected. A larger quantity will establish an excellent block, but the animals quite frequently die one to three hours later. It has been assumed that death was due to an interruption of the synapses on the phrenic nuclei which lie in the immediate proximity.

The Immediate Blood Pressure Changes.—It is a well established experimental fact that section of the cord in this region deprives the animal of a compensatory mechanism which is active in maintaining blood pressure in the face of adverse hydrostatic conditions. Thus elevation of the feet causes a maintained rise in blood pressure, while lowering of the feet causes a similar fall. This is in sharp contrast to the behavior of the normal animal, which after a short delay in each set of experimental conditions returns the pressure to the normal level. Therefore, it is important that the feet be elevated before the cord is cut and that this relative position be maintained for a number of hours after the operation. On several occasions, when ether was being used as an anesthetic, it was found that the lifting of the head and shoulders of the animal, even for the purpose of bandaging, caused an almost immediate respiratory stand-still. It then became a rule not to remove an animal from the operating board or change his position for from twelve to eighteen hours after the section.

Treatment of Collapse after Cord Section.—Despite the use of precautions, which have been outlined above, a state of collapse, in which the respirations were labored and shallow and the tissues cyanotic, sometimes followed immediately upon section of the cord. If now an injection of 22 mg. of caffein sodium benzoate be given into the ear vein, the respirations become more rapid, the color of the tissues improves and in a short time the animal's condition is satisfactory.

Period of Experimental Usefulness.—The animals drink water in eight to twelve hours and begin to eat food within twenty-four hours in satisfactory quantities, and in normal quantities after forty-eight hours. On the third day they can be regarded as in good experimental condition. In this condition they remain for six to fourteen days. Emaciation of the paralytic portion of the body is rapid in spite of apparently satisfactory feeding. This wasting has been noted by other observers. It is probably largely due to inadequate feeding. The animal in his helpless condition can easily eat greens of all

types, but it is difficult to devise a method of feeding him oats and similar foods which are more nutritious. A nasal discharge manifests itself as the forerunner of a terminal pneumonia. This pneumonia is less apt to occur if the animals be operated upon immediately on their entrance to the laboratory before they have had opportunity to become infected from the stock rabbits. The last animal operated on was in good condition after twenty days and was sacrificed at this time. The autopsy showed complete section of the cord and no pneumonia.

Technic in Detail.—A rabbit (weight 2 to 2.5 kilos) is injected intramuscularly with morphine (1.6 mg) and atropine (0.22 mg.). One hour later the
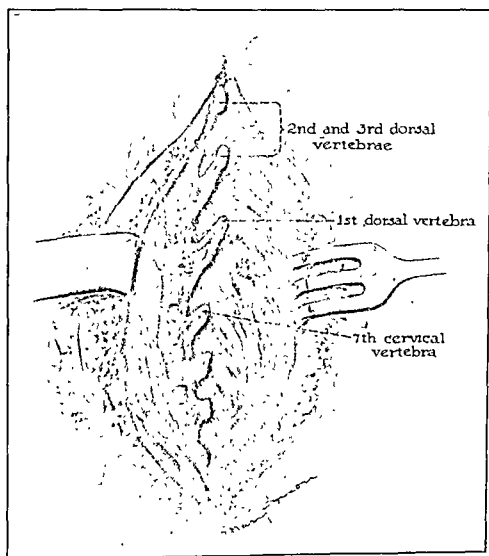

Fig 1

injection is repeated. Thirty minutes later (one and one-half hours after the first injection) the operation is begun. In the meantime the field has been shaved and prepared with alcohol. The animal is wrapped in a light blanket, placed on the left side with hind feet tied, front feet loose and the ears held by an assistant. The foot of the board is elevated four inches. Towels may now be clipped to the skin without any display of pain by the rabbit.

The skin is infiltrated with 2 per cent novocaine and the muscle layers blocked off by plunging a fine caliber needle to the lateral spinous processes. This catches the nerves shortly after their exit. For the entire operation 5 to 10 c.c. of the solution is usually sufficient. On exposing the spines of the

vertebrae, care should be taken to avoid the space lateral to the seventh cervical and the first dorsal vertebra. The vertebral vein is located here and it is quite easily damaged. A hemorrhage established may rapidly prove fatal. The seventh cervical spine may be located as follows. (See illustration.) The first two spinous processes which are long and of equal height are the second and third dorsal. The vertebra cephalad is about three-fourths as high and is of course the first dorsal. The next one marks the beginning of the cervical cord. Its height is so variable that it cannot be used as an identifying criterion. The spine is best removed by the use of a pair of sharp-pointed medium heavy scissors. These enable the operator to take short bites on either side of the process. If too much of the dorsum of the vertebra is removed, the intraspinal venous plexuses may be injured with a resulting rapidly fatal hemorrhage. The cord is now blocked with 0.3 to 0.4 c.c. novocaine. Immediately the area of the block is cauterized with a hot probe. This is done slowly so as to coagulate the tissues within the block and avoid extending the cauterization into unanesthetized territory. It is this extension which appears to precipitate the collapse (shock) in the animals. After the closure with catgut, external heat is applied as needed to maintain the temperature.

REFERENCES

[1]Tscheschichin, J.: Arch. f. Anat. u. Physiol., 1866, p. 151.
[2]Pflüger, E.: Arch. f. Physiol., 1878, xviii, 247.
[3]Nebelthau, E.: Ztschr. f. Biol., 1895, xxxi, 292.
[4]Schönborn, E. Graf.: Ztschr., f. Biol., 1911, n.s. 33, 209.
[5]Balcar, J. O., Sansum,. W. D., and Woodyatt, R. T.: Arch. Int. Med., 1919, xxiv, 116.
[6]Schmitt, E. C., and Sansum, W. D.: Calif. State Jour. Med., September, 1921, xix, 371.
[7]Freund, H., and Strasmann, R.: Arch. f. exper. Path. u. Pharmakol., lxix, 12.
[8]Krause, W.: Die Anatomie des Kaninchens; W. Engelmann Leipzig, 1884, 327.
[9]Ellenberger and Baum: Anatomie des Hundes, P. Parey, Berlin, 1891, 540.
[10]Sherrington, C. S.: Schaefer's Text Book of Physiology, ii, 853.
[11]Moore, Lillian: Am. Jour. Physiol., 1918, xlvi, 244.

FRACTIONATION OF THE PROTEINS OF RATTLESNAKE VENOM*

By WILLIAM H. WELKER, A.C., PH.D., CHICAGO, ILL.

IN 1904, John Marshall[1] announced that he had succeeded in separating the proteins of rattlesnake venom into toxic and nontoxic fractions. Up to the time of his announcement, none of the investigators had succeeded in getting nontoxic protein fractions from the venom. The principle of the method employed was the addition of saturated ammonium sulphate solution in such a manner that the concentration of ammonium sulphate in no part of the solution was ever markedly increased beyond that of any other part. This was accomplished by employing a dilute solution of the venom and very rapid mechanical stirring while adding saturated solution of ammonium sulphate.

Marshall was compelled to abandon further experimentation through accidental sensitization to the venom of the rattlesnake which has persisted to the time of the writing of this article. In transferring some newly acquired rattlesnakes from the box in which they had been transported, one of the snakes in the box struck and momentarily entangled its fangs in the wire netting covering of the box and ejected venom, some of which came in contact with one of Marshall's eyes. Intense perspiration within five minutes of the occurrence was the first symptom observed; the conjunctiva became decidedly distended with an extravasation of a yellowish serum which disappeared in about three days on treatment with cold compresses. This accident, however, resulted in a sensitization so marked that dried venom, or indeed a solution of venom, left exposed in the same room produced an intense disturbance in his air passages with the production of great quantities of a thick, tenacious mucus, the disturbance lasting two or three months.

In a personal communication to the writer, Marshall pointed out that the only way to obtain rapid and complete solution of the venom is to moisten the dried material with a few drops of one per cent sodium chloride solution and when completely softened to dilute with one per cent sodium chloride solution to the desired volume. If the venom shall not have been filtered, preferably through a very small, hardened filter paper, immediately after its procurement from the snake, a quantity of material in the dried venom, consisting of debris from broken down cellular tissue, will refuse to dissolve in any liquid. Pulverizing the venom renders a very large portion of the substance insoluble.

Marshall, in his communication, also states that in his experiments, the autopsies on the pigeons in which death occurred from the injection of the toxic fraction, showed the spleen intensely congested. The intestinal tract also was congested but to a less degree than the spleen.

Experimental.—In the work reported herewith, an apparatus was devised for purposes of stirring which could be driven by a small motor. In Experiment I, it was endeavored to redissolve the individual fraction and reprecipitate the same until the solution of the fraction showed no precipitation on either side of the limits of the fraction. This entailed very serious loss of protein material, because of a marked tendency of a given fraction to shift its precipitation limits. On account of this difficulty, the number of reprecipitations for the different fractions was reduced in Experiment II.

Experiment I.—Ten grams of dry rattlesnake venom were moistened with a small quantity of physiologic salt solution and when complete liquefaction had been effected, the solution was made up to one liter with distilled water and permitted to stand twenty-four hours under toluol. A white flocculent precipitate resulted. This method of precipitation was used for euglobulin because of the very gradual formation of the precipitate. The white flocculent precipitate was filtered off, dissolved in 10 per cent sodium chloride solution and precipitated by a saturation up to 33 per cent of ammonium sulphate. The precipitate was redissolved and reprecipitated in this fashion four times, including the first precipitation, then dissolved in a 10 per cent sodium chlo-

EXPERIMENT ON GUINEA PIGS

PROTEINS OF FRACTIONATION EXPERIMENT I

INJECTIONS MADE INTRAPERITONEALLY

	FRACTION	WEIGHT OF SUBSTANCE	WEIGHT OF GUINEA PIGS	REMARKS
A	(0 to 33 per cent)	10 mg.	270 gm.	No effect
B	(33 to 46 per cent)	10 mg.	300 gm.	No effect
C	(46 to 64 per cent)	10 mg.	350 gm.	No effect
D	(64 to 100 per cent)	10 mg.	365 gm.	No effect
E	(about 100 per cent)	5 mg.	235 gm.	No effect

Solutions of all fractions but A (0 to 33 per cent) were made in physiologic salt solution, and fraction A was made in 5 per cent sodium chloride solution in which it was only slightly soluble.

EXPERIMENT ON PIGEONS

PROTEINS OF FRACTIONATION EXPERIMENT I

INJECTIONS MADE INTO THE BREAST MUSCLES OF PIGEONS

	FRACTION	WEIGHT OF SUBSTANCE	WEIGHT OF PIGEONS	REMARKS
A	(0 to 33 per cent)	10 mg.	333 gm.	*
B	(33 to 46 per cent)	10 mg.	327 gm.	No effect
C	(46 to 64 per cent)	10 mg.	339 gm.	No effect
D	(64 to 100 per cent)	10 mg.	344 gm.	No effect
E	(above 100 per cent)	5 mg.	313 gm.	No effect

The solutions of these fractions were made in the same way as in the experiment on guinea pigs.

*No effects were noted except in the case of Fraction A. This pigeon did not appear abnormal until fourteen hours after the injection and was found dead, in rigor mortis, twenty-five hours after the injection.

ride solution, filtered and dialyzed free from ammonium sulphate and sodium chloride, centrifugalized and washed with distilled water until the washings gave no biuret test.

The 33 to 46 per cent fraction was precipitated four times including its initial precipitation. The solution of the third precipitate showed practically no precipitation at 33 per cent and practically none at 46 per cent of ammonium sulphate solution. The solution of the fourth precipitate was filtered and dialyzed free from ammonium sulphate and sodium chloride.

The 46 to 64 per cent fraction was precipitated three times, then dissolved and dialyzed free from ammonium sulphate and sodium chloride.

The saturated filtrate from the first precipitation of the albumin was dialyzed free from ammonium sulphate and sodium chloride.

Experiment II.—In a second experiment, two and one-half grams of dry rattlesnake venom were moistened with a small amount of physiologic salt solution and when complete liquefaction was effected, the solution was diluted to 250 c.c. with distilled water and permitted to stand overnight. The precipitate was filtered off, dissolved in 10 per cent sodium chloride, saturated to 33 per cent of ammonium sulphate, filtered, washed by centrifugalization with distilled water until free from soluble protein, and dried.

The 33 to 46 per cent fraction was precipitated from the filtrate of the first fraction by saturation to 46 per cent of ammonium sulphate solution. The precipitate was dissolved in water, the solution saturated to 33 per cent, permitted to stand overnight and the precipitate which formed filtered off.

EXPERIMENT ON PIGEONS
PROTEINS OF FRACTIONATION EXPERIMENT II
INJECTIONS MADE INTO THE BREAST MUSCLES OF PIGEONS

	FRACTION	WEIGHT OF SUBSTANCE	WEIGHT OF PIGEONS	REMARKS
*1	(0 to 33 per cent)	10 mg.	290 gm.	No effect
2	(33 to 46 per cent)	10 mg.	295 gm.	Pigeon showed signs of discomfort 4½ hours after injection: found dead, but warm, seven hours after injection
†2A	(33 to 46 per cent)			
3	(46 to 64 per cent)	10 mg.	300 gm.	Pigeon recumbent 4½ hours after injection: found dead, but still warm 7 hours after injection
‡3A	(0 to 33 per cent)	10 mg.	320 gm.	No effect
4	(64 to 100 per cent)	10 mg.	295 gm.	No effect
5	(above 100 per cent)	10 mg.	300 gm.	No effect

Solutions made in physiologic salt solution, except in the case of 3A, which was made in 10 per cent sodium chloride.

*Insoluble in 10 per cent salt solution.
†From the solution of the first precipitate at (46 to 64 per cent) saturation.
‡From the solution of the first precipitate at (33 to 46 per cent) saturation. Insoluble in water, soluble in 10 per cent sodium chloride solution.

EXPERIMENT ON PIGEONS
PROTEINS OF FRACTIONATION EXPERIMENT II
INJECTIONS MADE INTO THE BREAST MUSCLES OF PIGEONS

	FRACTION	WEIGHT OF MATERIAL	WEIGHT OF PIGEONS	REMARKS
2A	(33 to 46 per cent)	10 mg.	297 gm.	Dead three hours after injections
3	(46 to 64 per cent)	10 mg.	317 gm	Recumbent in five minutes after injection, gasping for breath 13 minutes after, dead 14 minutes after injection
3A	(0 to 33 per cent)	10 mg	318 gm.	Slight signs of discomfort 15 minutes after injection, later normal
*4A	(46 to 64 per cent)	10 mg.	327 gm.	No effect
5	(above 100 per cent)	10 mg.	292 gm	No effect

Solutions made in physiologic salt solution except in the case of 3A which was made in 10 per cent sodium chloride solution.

*Precipitate up to 64 per cent saturation of ammonium sulphate from second precipitation of albumin.

The filtrate was raised to 46 per cent saturation with ammonium sulphate solution. The precipitate was filtered off, dissolved and dialyzed.

The precipitate at 33 per cent of saturation was washed with 33 per cent saturated ammonium sulphate solution, dissolved in 10 per cent sodium chloride and dialyzed free from salts. The suspension of the precipitate was centrifugalized, the precipitate washed with distilled water by centrifugalization until free from soluble protein and dried. This material is indicated as "3-A" in the animal experiments.

The filtrate from the first precipitate of the 33 to 46 fraction was saturated to 64 per cent and the precipitate was filtered off, redissolved in water and saturated to 46 per cent. The precipitate at 46 per cent was filtered off, dissolved in water and dialyzed free from ammonium sulphate and is indicated as "2-A" in the animal experiments. The filtrate was saturated to 64 per cent with ammonium sulphate solution. The precipitate was dissolved in water and the solution dialyzed free from ammonium sulphate.

The filtrate from the 64 per cent precipitate was saturated with chemically pure, solid ammonium sulphate. This precipitate was dissolved in distilled water and the solution saturated to 64 per cent. The precipitate occur-

ring at this degree of saturation was filtered off, dissolved, dialyzed and dried. This fraction is indicated as "4-A." The filtrate was saturated with solid ammonium sulphate, the precipitate filtered off, dissolved and dialyzed free from ammonium sulphate and sodium chloride.

All the solutions of the precipitates were dried at 40° C. in the presence of toluol.

The saturated solution of ammonium sulphate was dialyzed free from ammonium sulphate for the purpose of securing any colloidal material not precipitated by full saturation with ammonium sulphate.

DISCUSSION OF RESULTS

In the toxicity tests made on products of the first fractionation experiment, it appears that practically all of the toxicity had been lost from the protein fractions. The fraction from 46 to 64 per cent which was found toxic by Marshall and which also showed high toxicity in the second experiment is nontoxic when purified by reprecipitations from its accompanying toxic principle. The slight toxicity of the euglobulin fraction can be explained on the basis of incomplete removal of water-soluble protein by washing. While the wash water showed no biuret-reacting substances, the fact that this fraction showed slight solubility after drying, would indicate that not all of the water-soluble material had been removed from it.

In the toxicity tests from the materials of the second fractionation experiment, the only fractions showing toxicity were first: the fraction precipitated by a saturation of 46 to 64 per cent and the 2A fraction which was the precipitate occurring between 33 and 46 per cent saturation in the first reprecipitation of the 46 to 64 fraction. In this experiment the euglobulin fraction when dried at 40° C. (after water-soluble proteins and electrolytes had been removed) became completely insoluble in 10 per cent sodium chloride. A similar observation has been made in this laboratory, in the fractionation of blood serum in another investigation which will be published shortly. Rippel, Ornstein, Carl, and Lasch[2] have recently reported a similar observation.

CONCLUSIONS

1. The results of these experiments confirm the work of Marshall in showing the feasibility of separating rattlesnake venom into toxic and nontoxic protein fractions.

2. They show that the euglobulin on being dried after the removal of water-soluble proteins and electrolytes is no longer soluble in 10 per cent sodium chloride.

3. There is an indication that on repeated reprecipitation of the toxic fraction, the toxic principle contained in the fraction may be removed.

Further work is in progress on the toxic fraction, with a view of studying the nature of the toxic substances of the venom.

My thanks are due Professor Marshall for his kind advice and suggestions in connection with this investigation and also for the rattlesnake venom used in the investigation.

REFERENCES

[1] Science, n. s., 1904, xix, 715.
[2] Ripple, Ornstein, Carl and Lasch: Ztschr. f. Hyg. u. Infectionskrankh., 1922, xcvii, 188.

LABORATORY METHODS

QUANTITATIVE WASSERMANN TEST*

By E. H. RUEDIGER, M.D., Los Angeles, Calif.

I BELIEVE that the Wassermann test is here to stay for some time to come and consider it our duty to develop it to the highest degree of efficiency. In order to develop it intelligently we must agree on some sort of a unit of measure and do strictly quantitative work. Without a unit of measure quantitative work cannot be done, and without quantitative work we do not get the information we ought to have, and we do not give the clinician and the patient the information for which they long and which they should obtain.

Many laboratories are still doing no more than qualitative work with the Wassermann test. They never report more than 4+ (++++). This means at least 4+, but the serum tested may titrate 50+, 100+ or even 1,000+. Supposing that a patient who would give 1,000+ result with the Wassermann test and has a report which says 4+ or ++++, is put on treatment and is treated for a year and the reports on the Wassermann test still read 4+, the patient and the physician are sure to be discouraged because it shows no improvement. Entirely different is the situation when quantitative work is done. If the first report reads 1,000+, treatment is urgently indicated, and if the second report, about six months after beginning of treatment reads 400+, patient and physician receive encouragement.

If the third report, about a year after the first report, reads 200+ patient and physician receive new encouragement, moreover they will soon understand that under such conditions negative results are not to be expected within a few months, and frequently treatment must be continued for several years, and that many chronic cases never become negative.

For several years I have been doing quantitative titration on every serum that was submitted, and the results have been very satisfactory. The following report shows some of the results obtained on cases that could be followed up for some time.

METHOD

Human Serum.—All human sera were heated to about 56° C. for thirty minutes and were mixed with pure, sterilized glycerol so as to have a mixture containing fifty per cent glycerol. The first dilution I usually make 1:4 by adding one part of human serum to three parts of a mixture containing two

Transfer the tubes from the cold water-bath to a warm water-bath at 37° C. and from five to ten minutes later add 0.4 c.c. of amboceptor-corpuscle mixture to each tube. Return the tubes to the warm water-bath for one hour during which time they should be shaken at intervals of fifteen minutes. Remove the tubes from the warm water-bath, let them stand at room temperature for about one hour, and read the results.

Reading Results.—In reading results complete inhibition of hemolysis in a tube is called 1÷ or one unit fixation and this is multiplied by the dilution of the human serum.

Example.—

No. of Tube	1	2	3	4	5	6
Dilution of Human Serum	1:4	1:4	1:12	1:36	1:108	1:324
No. of Test						
1 Reading	0	+	+	+	0	0= 36+
2 "	0	+	+	+	+	0=108+
3 "	0	+	+	+	+	+=324+

If the last antigen tube does not show the limit of fixation the serum is titrated further, beginning with a higher dilution.

Example.—

No. of Tube	1	2	3	4	5	6
Dilution of Human Serum	1:100	1:100	1:200	1:400	1:800	1:1600
No. of Test						
1 Reading	0	±	+	+	0	0=400+
2 "	0	+	+	+	+	0=800+

The following report shows the results obtained on seven syphilitic patients who were under observation for varying lengths of time.

CASE 1.—This was a chronic syphilitic who came under observation in November, 1920. The first Wassermann test, with blood obtained in November, 1920, gave 150+. A second Wassermann test, in June, 1921, gave 70+. In November, 1921, the Wassermann test showed 40+, in February, 1922, it showed 50+, in February, 1923, it showed 20+, in May, 1923, it had gone up to 30+ and in September, 1923, it had risen to 50+. See Chart 1.

CASE 2.—This was a child about ten years old who had congenital syphilis. At this time the parents sought treatment because the eyes of the child had become very bad. The first Wassermann test was done in March, 1922, and showed 200+. In July, 1922, the Wassermann test showed 70+ and in November, 1922, it was 20+. See Chart 2.

CASE 3.—A child about eight years old had a very bad throat which did not respond to ordinary treatment. The first Wassermann test was done in April, 1922, and showed 500+. Antisyphilitic treatment was given and the throat improved rapidly. In July, 1922, the Wassermann test showed 400+, in October, 1922, it showed 300+ and in January, 1923, it showed 200+. In this case the objective and subjective symptoms concerning the actual lesion disappeared rapidly, and the quantitative Wassermann test showed decided improvement, but an old-fashioned qualitative Wassermann test might have shown ++++ with each of these four tests. See Chart 3.

CASE 4.—Patient 4 of this series was a child about seven years old with congenital syphilis who was brought for treatment because he had become nearly blind. The first Wassermann test was done in July, 1922, and showed 400+. The second test was done in October, 1922, and was 40+; by April, 1923, it had risen to 100+ again, and in September, 1923, it was found to be 20+. See Chart 4.
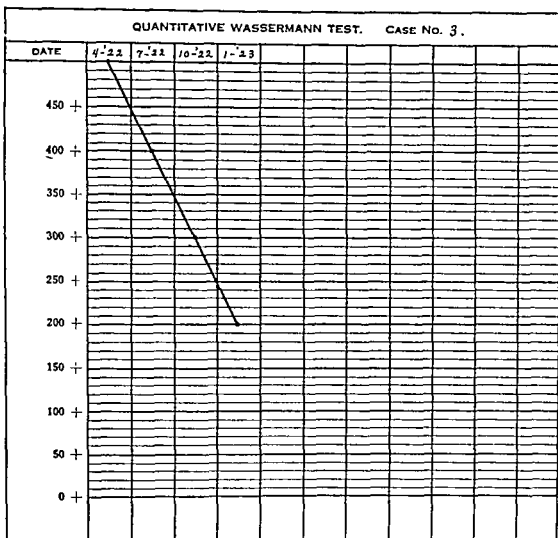
Chart 3.
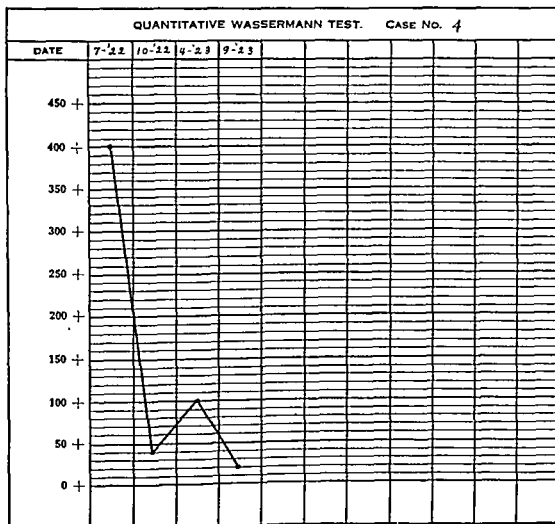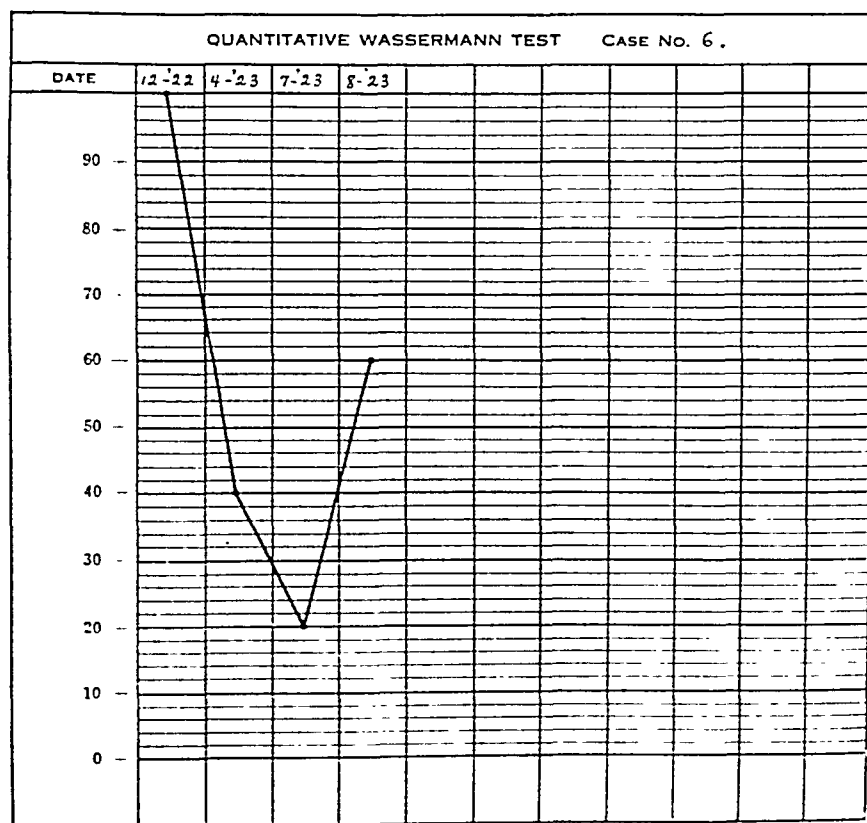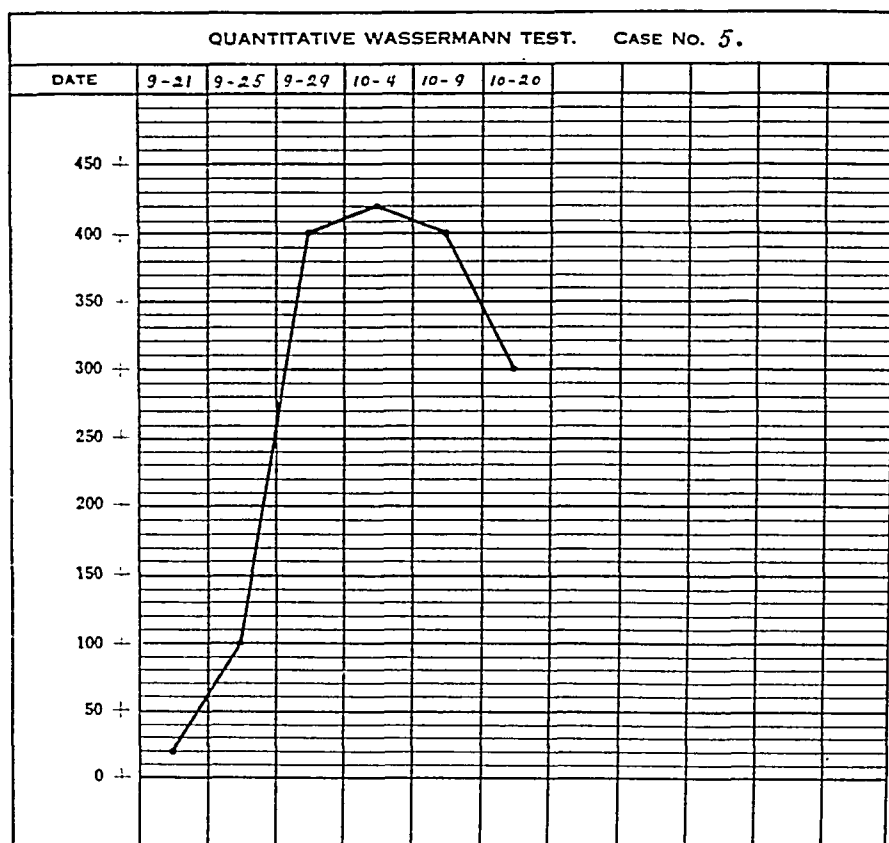

Chart 4.

Chart 5.

Chart 6.

CASE 5.—Case No. 5 is an extremely interesting one. A young man consulted a physician because of a venereal sore which had appeared a few days before. The physician convinced himself that the lesion was not a syphilitic lesion, gave the patient something to be used locally and asked the patient to come back after a few days. The patient returned, and the sore was bigger than it had been at the first visit. After reprimanding the patient for not having used the medicine, the physician sent him to the hospital where a Wassermann test was done on September 21, and was found to be 20+. This did not change the physician's views; he still considered the present lesion nonsyphilitic. On September 25 a second Wassermann test was done and this one showed 100+, but without making any impression on the physician because he had excluded syphilis. On September 29, the Wassermann test showed 400+, the patient had a profuse purple rash and antisyphilitic treatment was begun. The rash faded rapidly and the sore began to heal. Was-
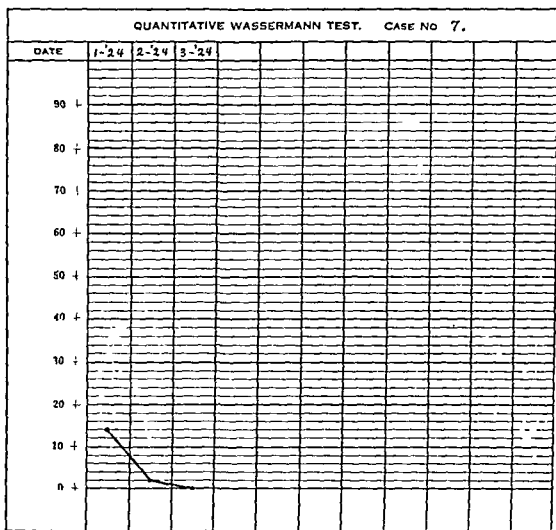

Chart 7.

sermann tests done on October 4, October 9, and October 20, showed 420+, 400+, and 300+ respectively. See Chart 5.

CASE 6.—A woman consulted her physician because she was rapidly losing her hearing. A Wassermann test was done in December, 1922, which showed 100+. She was given antisyphilitic treatment. In April, 1923, the Wassermann test showed 40+; by July, 1923, it had fallen to 20+; treatment was discontinued and the patient was instructed to return within a month. In August, 1923, the Wassermann test showed 60+ and the patient was advised to take treatment regularly for some time to come. See Chart 6.

CASE 7.—This case demonstrates the importance of showing improvement even with the Wassermann test. A young man contracted syphilis, went to his physician and was put on antisyphilitic treatment. The first Wassermann test at that time was reported as strongly positive, ++++. The treatment was taken regularly and Wassermann tests were done at intervals of a few months but the reports on them always read "strongly positive,

+++.'' The patient became disgusted with this report and went to another physician for further examination and treatment. The second physician asked me to do a Wassermann test on this patient in January, 1924, and I reported, "positive, 12+," and explained the difference between qualitative and quantitative Wassermann test. The patient was well pleased, he received no treatment but was instructed to return in a month. In February, 1924, he reported for another Wassermann test which was done and found to be 2+. This time the patient was greatly pleased; he received no treatment but was instructed to report for another Wassermann test after one month. In March 1924, I did the third Wassermann test on this patient and found it negative. This patient has been instructed to come here for Wassermann test once or twice a year to find out whether it remains negative or becomes positive again, and some time in the future I may be able to report on this case again. See Chart 7.

SUMMARY

Quantitative titration in the Wassermann test is as satisfactory as quantitative titration of typhoid agglutinating serum, diphtheria antitoxin or tetanus antitoxin. A quantitative Wassermann test gives far more information than does a qualitative Wassermann test. In early doubtful cases a rising titre with the Wassermann test may be considered diagnostic of syphilis. During treatment, a falling curve with the Wassermann test gives valuable information and encouragement to patient, physician and laboratory worker.

(For discussion, see p. 303.)

A NOTE ON THE PREPARATION OF COLLOIDAL GOLD SOLUTION BY THE MELLANBY-ANWYL-DAVIES TECHNIC

By Russell L. Haden, M.D.*

THE preparation of colloidal gold is an unsatisfactory procedure for many laboratories. The technic of Miller, Brush, Hammers and Felton,[1] which is employed by most workers, is tedious and time consuming. Different batches of the solution, even when made the same day, will often vary quite widely in reaction. Good results in the test depend largely upon the correct reaction of the reagent. An acid solution is too sensitive, and an alkaline one is not sensitive enough.

Mellanby and Anwyl-Davies[2] have recently described a new method of preparation which is very simple. They state that the solutions so made are always neutral in reaction. The solutions that I have prepared by their technic, however, have always been too acid, so that a luetic type of curve is obtained even with a normal spinal fluid. This result is probably due to a difference in the acid content of the gold chloride used by the English workers. In attempting to overcome this difficulty I have added potassium carbonate without very satisfactory results. Uniformly good solutions can be made, however, by the addition of potassium hydroxide. The optimum amount to be added can be determined by a preliminary test using the reagents which are to be employed in the preparation of the reagent.

Reagents.—(1) One per cent gold chloride solution made by dissolving a 15 grain tube of Merck's gold chloride in 100 c.c. of freshly distilled water.

(2) One per cent potassium oxalate solution prepared by dissolving 1 gram of *neutral* potassium oxalate in 100 c.c. of freshly distilled water.

(3) One per cent solution of potassium hydroxide made by dissolving 1 gram of potassium hydroxide (c.p. purified by alcohol) in 100 c.c. of freshly distilled water.

Preliminary Test.—Set up six clean test tubes in a rack and into each run 1 c.c. of the 1 per cent gold chloride. Add 1 per cent potassium hydroxide as follows: Tube No. 1, 0 6 c.c., No. 2, 0.5 c.c., No. 3, 0.4 c.c., No. 4, 0.3 c.c., No. 5, 0.2 c.c., and No. 6, 0.1 c.c. To each tube add 2 drops of a 1 per cent solution of phenolphthalein in 95 per cent alcohol. The tube containing the largest amount of alkali will remain clear and react alkaline. The other tubes show varying degrees of turbidity, the one having the optimum reaction being the most turbid. This is usually the tube containing 0 3 c.c. alkali. One c.c. of 1 per cent neutral potassium oxalate is now added to each tube. Reduction will begin quickly, starting in the tubes having the optimum reaction. Within five minutes, tubes 4, 5 and 6 are completely reduced as indicated by a dense black precipitate; tube 1 is alkaline and clear; tube 2 is turbid and of a lead color due to partial reduction. The optimum amount of the potassium hydroxide to use in the preparation of the colloidal gold solution is the largest amount which can be added and still have complete reduction take place in five minutes as shown by the preliminary test. With the different samples of Merck's gold chloride which we have tried, this is usually 0.3 c.c. of the 1 per cent solution for each c c. of the gold chloride solution. Colloidal gold solutions made with this amount of alkali are uniformly neutral. If only 0.2 c.c. is added the reagent is too acid, and with 0.4 c.c. it is too alkaline.

The amounts given may vary with different batches of gold chloride but the principle of correction is the same with all.

Preparation of Reagents.—The exact technic for the preparation of the reagent follows: To 100 c c. of distilled water 1 c.c. of the 1 per cent potassium oxalate solution is added, and the fluid is heated to the boiling point. While boiling, 1 c.c. of the gold chloride solution, to which the requisite amount of alkali (i.e., a total of 1.3 c.c. if 0.3 c.c. is the optimum amount of alkali) has been added, is run in drop by drop. A clear red, neutral solution of colloidal gold is quickly obtained.

All glassware should be clean and of the hard variety such as Pyrex. The water must be doubly distilled, the second distillation being made immediately before use. Since city water supplies often contain chloride, it is preferable to start with spring or rain water. The stock solutions appear to keep indefinitely. It is an advantage to add 1 c.c. of 10 per cent potassium permanganate and 1 c.c. of a saturated solution of barium hydroxide to each 2 liters of water before the second distillation.

REFERENCES

[1]Miller, S. R., Brush, N. D., Hammers, J. S., and Felton, K. D.: Bull. Johns Hopkins Hosp., 1915, xxvi, 391.
[2]Mellanby, J., and Anwyl-Davies, T.: Brit. Jour. Exper. Path., 1923, iv, 132.

FURTHER STUDIES WITH CRESYLECHT VIOLET INCLUDING A REPORT OF MY SIX-SECOND METHOD FOR STAINING TISSUES*

By B. G. R. WILLIAMS, M.D., PARIS, ILLINOIS

ASSISTED BY E. M. WILLIAMS, B.S., PARIS, ILL., AND SR. M. TIBURTIA, TERRE HAUTE, IND., AND MISS MARGARET DRUMMY, TERRE HAUTE, IND.

LET us see in fancy, a method for tissue staining suitable both to fresh and fixed materials, easily completed in six seconds and with the following reactions resultant, viz.:

Nuclei.—Dark blue.

Cytoplasm.—Light blue or violet.

Blood.—Pea green.

Muscle.—Greenish blue or blue (according to thickness of sections).

Fat.—Bright, translucent lemon-yellow or orange.

Fibrils.—Pink to magenta.

Fibrin.—Sky-blue.

Calcification.—Dirty blue.

Caseation.—Granular-blue.

Metachromatic reactions for mucin, colloid, hyalines, etc., etc.

Such a staining method should prove of great value, particularly in surgical pathology.

METHODS IN GENERAL USE

Various polychrome methylene blues have been used. Their shortcomings have been many. They will in a general way, stain nuclei blue and stroma a bluish-red; but metachromasia stops there. The blues stain fairly rapidly but not sharply, giving only general outlines and little detail. They aid in some diagnoses; but in no manner approach the results suggested above. Of these blues, the best in my opinion, is that of Goodpasture; that of Wilson is also with some merits while that of Terry is more easily prepared but gives the poorest results of this class of solutions.

CRESYLECHT VIOLET PROPOSED

For a number of years, we have studied the possibilities of cresylecht violet and about a year ago, made a preliminary report.[1] More recently, however, we have made a number of improvements in our solutions and technic. While the work may be said to be still in an experimental stage, the method given below has yielded results closely approximating those listed above. This I have termed my six-second method. While demonstrating this method

to some pathologists recently, they insisted that it should be published and other workers given opportunity to follow up the work in the future.

SOLUTION USED IN THIS METHOD

A number of cresylecht violets and dyes supposed to be cresylecht violet have been submitted to me by the Commission on Standardization of Biological Stains. Of these there is but one which gives the results listed above and this is made by the National Aniline and Chemical Co., Inc.* Some of the foreign dyes show metachromasia but they stain feebly and fail to stain fat, erythrocytes, etc. Their reactions resemble somewhat the polychrome blues.

I make up a concentrated solution of the dye in full strength, white water formalin. This formalin I have secured from the Will Corporation. Other formalins are likely to give poor results because of their high content of methyl alcohol. This solution is filtered and kept tightly corked. It seems to be permanent, and I am using some which was prepared six months ago.

This solution differs in many respects from that described in my former communication. The other solution was dark blue; but this solution appears opaque and almost black in reflected light. It is in fact so opaque that it cannot be used in watch glasses without losing the sections. I have tried lighting my staining tables but only thin films of the stain transmit this light, not enough for practical staining. The color of such thin films is dark blue. It seems that the dye is soluble to greater concentration in this reagent than in water. Moreover the solution is less "greasy" and does not adhere to the glassware as did the one formerly described.

FIXATION

Unfixed tissues may be stained. If time permits, they may be fixed in 10 per cent white water formalin. Alcohols are to be avoided at all costs inasmuch as they dissolve out fat and blood and interfere with freezing methods and staining.

SECTIONING

Frozen sections are used. Thickness of sections will depend upon individual taste. For beautiful preparations the thin section would be preferable; ten to fifteen microns in thickness is better for most tissues in surgical pathology. This thickness will enable the pathologist to study relations "up and down" by changing focus slowly, a point which I have always emphasized but which most men overlook in the attempt to secure thin sections. Moreover when dealing with fresh materials, the sections when thin often roll, wrinkle, ball and cohere, delaying the work. If staining is delayed, the sections may be kept in a vial containing 10 per cent formalin. Many months later, they may be stained by this method.

STAINING

I have stated that the solution is very opaque so that I have done away with watch glasses. The section is spread upon the lifter; excess water is drained

off but the section is not blotted. The stain is added by means of a pipette. When the section is covered (and one drop will suffice with a small section), approximately six seconds are counted. Then the lifter with section is dipped into a large basin of water* and the section washed free into the water and paddled about gently with the lifter. Lengthening this staining time does not seem to result in overstaining and may be desired in some instances but six seconds will suffice. The solution is corked to avoid evaporation. About one to two seconds will be sufficient for washing section if there is plenty of water.

EXAMINATION

The water-mount may be examined at once. We use the larger slide (2 × 3 in.) and cover by the long rectangle because a number of sections may be glanced at simultaneously if desired.

PERMANENT MOUNTS

The method was designed for diagnostic purposes where haste is imperative and permanent mounts are not satisfactory. I have, however, tried out several methods of mounting, and find that preparations in levulose will be suitable for reference or teaching purposes for a few days. When we find a particular section which we would like to mount, we wash out the cresylecht violet with alcohols and restain with eosin and hematoxylin, though of course we sacrifice many details in so doing. Although designed only for rapid diagnostic work, many pathologists have insisted that I also devise some method for permanent mounts that these preparations may be used for teaching. However, thus far I am compelled to admit failure.

RÉSUMÉ

In our hands, the method has given some beautiful pictures and is suggested for further study by pathologists. It may be possible to improve upon this solution, but even in its present status, it is so superior to the polychrome methylene blue methods that they may well be abandoned and attention centered on cresylecht violet.

At first thought it might seem that we are asking too much of an oxazin in the matter of the metachromasia listed above: I do not think so. I have always felt that such reactions could be secured with sufficient effort and study, and this has been the reason for my investigations. It might seem farfetched practicability to stain fat with other elements, and so it seemed to me when I discovered this property of cresylecht violet in 1921. But I have learned that it often explains "spaces" overlooked in some preparations. Thus in some mixed connective tissue tumors, this has been proved. Quite recently I noted what seemed to be a fat metaplasia in a cervix uteri, and which would not have been revealed by other methods. It also serves to show diffuse metastases in adipose tissue of omentum, etc., which might otherwise be overlooked.

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*A large, shallow dish is suitable. I prefer a Pyrex cake dish or baking dish about 8 x 8 in.

The same may be said in regard to the pea-green blood cells and the metachromasia of muscle versus nuclei in myopathic lesions of appendix. The same is true in some granulations in the differentiation of fibrils and fibrin.

REFERENCE

¹Williams, B. G. R.: Cresylecht Violet, A Rare Dye, JOUR. LAB. AND CLIN. MED., viii, No. 4.

THE RED CORPUSCLE SUSPENSION FOR THE KOLMER-WASSERMANN REACTION*

By C. E. REYNER, B S , DETROIT, MICHIGAN

IN the standardized Wassermann technic, proposed by Kolmer[1, 2] and used in this laboratory for two years, it has been found that the 2 per cent red corpuscle suspension of sheep's blood at times hemolyzes while standing overnight in the ice box. We use sheep's blood, secured from a slaughter house, in a solution containing 1.5 per cent sodium citrate and 0.85 per cent sodium chloride and it is frequently three or four days old when used. When hemolysis occurs in the red cell suspension, while standing overnight in the ice box, the positive results are not satisfactory to read and are weaker on account of the apparently increased fragility of the cells. This difficulty has been entirely overcome by means of the well-known fact that red cells hemolyze more rapidly when in dilute suspension than when concentrated. The method finally adopted follows:

The cells are washed with physiologic saline in the manner described by Kolmer and Brown.[3] Centrifuge the citrated blood without dilution, remove the supernatant fluid and add 3 to 5 volumes physiologic saline and centrifuge again; remove the supernatant fluid and add 3 to 5 volumes of the saline, centrifuge and remove the supernatant fluid. For measurement, the washed cells are packed into 15 c.c. graduated centrifuge tubes by centrifuging for five

TABLE I

Days old	1	2	3	4	5	6	7	8
2% Suspens	N.H.	N.H.	T.H.	P.H.	M.H.	* *	* *	* *
Packed cells Formal.	N.H.	N.H.	N H.	N.H.	N.H.	N.H.	P.H.	M.H.
2% Susp. Packed cells Form.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	P.H.
	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	N.H.	T.H.

N.H.—No Hemolysis.   T H.—Trace of Hemolysis.   P.H.—Partial Hemolysis.   M.H.—Marked Hemolysis.

TABLE II

Days old	1	2	3	4	5	6	7	8
No Preserv.	B.R.	B R.	B.R.	B.R.	D.R.	P.	M.H.	M.H.
Formalized	B.R.	B.R.	B R.	B.R.	B R.	B.R.	B.R.	B R.

B.R.—Bright Red.   D.R.—Dark Red.   P.—Purple.   M.H.—Marked Hemolysis.

TABLE III

Days old	1		2		3		4		5		6		7		8	
	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.	I.H.	C.H.
No preserv.	.800	.520	.800	.520	.800	.520	.800	.520	.800	.560	.800	.560	.800	.560	.840	.560
Formalized	.760	.520	.760	.520	.760	.520	.760	.520	.800	.520	.800	.520	.800	.520	.840	.520

I.H.—Initial Hemolysis.   C.H.—Complete Hemolysis.

minutes at 2,000 R.P.M. The tops of the cell columns are marked with a wax pencil and all tubes of packed cells are put away in the ice box except one which is used to prepare the cell suspension for the titrations. In the morning, when the series of ''set ups'' is ready for the final incubation, the packed cells are simply diluted with physiologic saline to make a 2 per cent suspension. The cells, kept in this way in the ice box overnight, have been found to hemolyze very little, if any, even when the cells are several days old.

When the dilute 2 per cent suspension of cells was kept overnight hemolysis was observed when the blood was three days old, whereas, when the packed cells were kept in the ice box overnight and then diluted to 2 per cent with 0.85 per cent saline, no hemolysis was observed on the sixth day. (See Table I.)

The apparently increased fragility of the cells kept overnight in a 2 per cent suspension is not borne out by fragility tests. For four days the initial and complete hemolytic points of the red cells remained constant at 0.800 per cent and 0.520 per cent sodium chloride respectively. It retained the levels of .800/.560 for three days and, on the eighth day, it was .840/560. (See Table III.)

When a 2 per cent cell suspension, prepared from sheep's blood six days old and permitted to stand in the ice box overnight, is compared with a suspension prepared from packed cells kept overnight in the ice box, a marked difference is observed.

THE USE OF FORMALIN AS A PRESERVATIVE OF RED CORPUSCLES

Kolmer and Brown[3] suggest the use of formalin as a preservative of red corpuscles. It is shown that a 2 per cent suspension of formalized sheep's corpuscles did not hemolyze while standing overnight in the ice box until the cells were over eight days old. Keeping packed formalized cells overnight did not show hemolysis until the cells were eight days old and then only a trace of hemolysis was observed. (See Table I.)

A comparison of the gross appearance of plain and formalized sheep's blood, as shown by Table II, reveals the fact that plain blood remains a bright red color for four days and then begins to get dark until, on the sixth day, it has a purple color and is totally unfit for use, and the formalized blood retaines its original bright red color for the entire period of eight days. The hardening of the blood cells with formalin has a slight effect on the fragility of the cells, as indicated by Table III, in that it decreases their fragility.

Difficulty, due to the hemolyzing of cells kept overnight in

the ice box while in dilute suspension, may be avoided by keeping the packed cells overnight and diluting just before use.

Formalizing sheep's cells enables one to keep them for a week or longer. It has been found that even formalized sheep's cells are more satisfactory if kept overnight packed, instead of in a 2 per cent suspension.

REFERENCES

[1]Kolmer, J. A.: A New Complement-Fixation Test for Syphilis Based upon the Results of Studies in the Standardization of Technic. Am. Jour. Syph., 1922, vi, 82.
[2]Kolmer, J. A.: Quantitative Complement-Fixation Test in Syphilis, Am. Jour. Syph., 1922, vi, 496.
[3]Kolmer, J. A., and Brown, C. P.: The Red Corpuscle Suspension for the Wassermann Reaction, Am. Jour. Syph, 1919, iii.

ON THE NONPROTEIN SULPHUR OF THE BLOOD*

By MAX KAHN, M.A., M.D., PH.D., AND ROBERT S. POSTMONTIER, B.S.,
NEW YORK

FOR the past several years,† an attempt was made in our laboratory to study the nonprotein sulphur fractions of the blood. We have finally adopted the following methods.

Total Nonprotein Sulphur of Blood.—Ten c.c of oxalated blood collected in the usual way from an arm vein, are treated with 90 c.c. of 2½ per cent trichloracetic acid to precipitate the total blood proteins, according to the method of Greenwald. After letting stand for half an hour, 80 c.c. of the filtrate are evaporated over a hot water-bath to half its volume, 10 c.c. of Benedict's sulphur solution are added, and the sulphur oxidized to sulphate, which is then determined gravimetrically as barium sulphate, according to Benedict's method.

Total Sulphate Sulphur of the Blood.—The method of Vansteenberghe and Bauzil was used. The reagents necessary are: Barium chloride, 12.2 gm. crystallized salt per liter; N/10 hydrochloric acid; 15 per cent sodium carbonate solution; 0.1 per cent aqueous solution of methyl orange; 10 per cent ammonium chloride solution; 36 per cent acetic acid solution. Ten c.c. of oxalated blood are treated with 40 c.c. of the ammonium chloride solution, and 1 c.c. of acetic acid added. The liquid is heated to boiling with stirring to coagulate the albumin. The liquid is then filtered, the filter washed once or twice, and the filtrate evaporated down to 10 c.c. It is then acidified with hydrochloric acid, and exactly 5 c.c. of the barium chloride solution added. The liquid is boiled for one minute, centrifuged, or filtered through talc, and an excess of the sodium carbonate solution added to the filtrate. The whole is then centrifuged or filtered, and the precipitate of barium carbonate washed

until the washings are neutral to litmus. The precipitate is then washed into a beaker, 10 drops of methyl orange solution added, and titration made with the N/10 hydrochloric acid. The end-point must match the tint obtained when adding ten drops of indicator to 0.5 c.c. N/1 hydrochloric acid in a volume of water equal to that used in the experiment.

The results that we have obtained by this method are extremely interesting from the clinical point of view. The figures should be considered as approximate rather than exact, and we would rather have the method considered as clinical-chemical than as purely chemical.

CARCINOMA

	Total Sulphur as SO_4	Inorganic and Etheral Sulphur as SO_4
1. Stomach	8.1	6.4
2. Stomach	10.2	8.5
3. Uterus	9.4	7.7
4. Uterus	9.5	7.2
5. Breast	7.5	5.2
6. Breast	5.6	4.1
7. Esophagus	11.0	8.3

SKIN DISEASES

	Total Sulphur as SO_4	Inorganic Sulphur as SO_4
1. Acne	3.7	2.1
2. "	2.5	1.9
3. "	2.8	2.2
4. Eczema	4.2	3.5
5. "	2.9	1.9
6. "	2.5	1.8
7. Psoriasis	7.3	6.7
8. "	7.4	6.2
9. "	6.4	5.4
10. "	8.2	7.2
11. "	5.6	4.2

CHRONIC INFECTIONS

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Tuberculosis	7.2	5.9
2. "	7.5	6.2
3. "	8.9	7.1
4. "	11.2	9.3
5. "	4.7	3.2
6. Syphilis	4.8	3.1
7. "	9.2	8.1
8. "	8.5	7.4
9. "	8.6	7.2
10. "	7.7	6.4

ACUTE INFECTIONS

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Typhoid	3.2	2.2
2. "	1.9	1.4
3. "	1.7	1.3
4. "	2.8	1.9
5. "	3.5	2.8
6. "	3.4	3.1
7. Pneumonia	3.7	2.8

ACUTE INFECTIONS—CONT'D

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
8. Pneumonia	5.8	4.2
9. "	8.6	7.5
10. "	8.4	7.8
11. "	3.2	2.6
12. "	2.9	2.3
13. "	4.3	3.5
14. Typhus	4.4	3.7
15. Septicemia	3.8	2.9
16. "	2.9	2.4

METABOLIC DISEASES

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Diabetes	7.5	6.6
2. "	12.3	9.7
3. "	4.2	3.7
4. "	5.7	4.2
5. "	5.9	5.1
6. "	6.8	6.1
7. "	7.4	6.6
8. "	8.7	7.2
9. "	4.9	3.7
10. "	4.5	3.5
11. Gout	4.3	3.8
12. Basedow	7.2	6.5
13. "	7.5	6.2

BLOOD DISEASES

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Secondary Anemia	3.7	3.1
2. " "	2.9	2.2
3. " "	2.8	2.1
4. Pernicious Anemia	7.5	6.6
5. " "	8.7	7.8
6. Chlorosis	2.2	1.8
7. Acute Leucemia	8.5	7.7
8. Hodgkin's Disease	2.9	2.2
9. " "	3.1	2.7
10. Myelogenous Leucemia	5.6	5.1
11. " "	5.7	5.0
12. " "	6.2	5.5
13. Lymphatic Leucemia	3.9	3.1
14. " "	3.8	2.9
15. " "	2.6	1.9
16. Von Jaksch Anemia	2.2	1.7
17. Hemorrhage	2.7	1.5

RENAL DISEASES

	Total Sulphur as SO_4	Sulphate Sulphur as SO_4
1. Acute Nephritis	14.3	12.1
2. " "	11.2	9.3
3. " "	15.7	11.2
4. Chronic Parench. Nephritis	10.3	8.4
5. " " "	2.5	1.8
6. " " "	7.8	6.2
7. " " "	6.2	5.9
8. Chr. Interstitial Nephritis	2.7	1.9
9. " " "	2.9	1.8
10. " " "	8.5	7.3
11. " " "	8.6	7.4

less specifically so stated $\left\{\begin{array}{l}\text{above.}\\\text{on main label.}\end{array}\right\}$," Two alternate forms of wording are used, as just indicated, because two forms of label are furnished by the Commission, one of which becomes the main label on the package, while the other is to be used in addition to the main label.

Certain stains have been put on the market labeled "Certified," but without the Commission label. Such labeling is of no value to the purchaser, as it merely indicates certification by the manufacturer himself.

Certification of stains by this batch method has to proceed slowly as specifications have to be worked out for each stain considered; but the following stains have already been put on the certification basis: Methylene blue, basic fuchsin, acid fuchsin, hematoxylin, safranin O, and pyronin. The companies to whom certification for some or all of these stains has been issued are: Empire Biochemical Co., Hartman-Leddon Co.; MacAndrews and Forbes, National Anilin and Chemical Co. and Providence Chemical Laboratories. Some of these companies do not carry all the previously mentioned certified products; but the majority of the dealers in general laboratory supplies plan to carry the certified stains as fast as certification is issued. The dealers known at present to be cooperating in this way are: Braun Knecht Heimann Co., Central Scientific Co., Denver Fire Clay Co.. E. F. Mahady Co., E. H. Sargent and Co., Scientific Materials Co., Arthur H. Thomas Co., and the Will Corporation. Others may also carry these stains; and if so the Commission will be glad to have its attention called to their omission.

The chairman of the Commission is always glad to answer questions concerning stains. Considerable information has accumulated as the result of the investigations already carried on, and often a specific question can be answered so as to show a prospective purchaser just where to go for the particular stain he wishes. The chairman's address is: Agricultural Experiment Station, Geneva, N. Y.

TRANSACTIONS

THIRD ANNUAL CONVENTION AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, ROCHESTER, MINNESOTA

June 5-7, 1924

(Continued)

Dr. Frederick E. Sondern, New York, Chairman, gave a **Provisional Report of Committee on Laboratories.**

In the course of the winter, your President appointed a committee, the object of which was to consider the inefficient private laboratory. The members of this committee live in all parts of the United States, and it was difficult, or really impossible, to get a meeting of this committee. Those that I could get together did meet in New York, so this, if we may call it a report, is one framed by a relatively small number of the committee. There are in this report certain recommendations. They may be considered rather radical by many; and in order that the members of the Association might have an opportunity to think them over and talk them over with one another, we requested our President to let me present this matter now, and then we can take it up at the regular business meeting on Saturday to do with it as you think best.

It is an established fact not only in this country, but also overseas, that a large amount of clinical laboratory work is being done by persons absolutely unqualified by training and experience. This state of affairs has during recent years engaged increasing attention particularly of those interested in public health, and a remedy is eagerly sought.

Practically speaking, clinical laboratory work is the result of a demand of the physician for aid in diagnosis, and were physicians as a class, exacting in the laboratory service rendered, the existing evil would soon come to an end.

These constantly increasing justified complaints relative to inefficient and misleading laboratory service, particularly in our larger cities, must have the serious attention of such bodies as our Association, and a remedy should be found to make very probable legislative control unnecessary or to guide such control if in public opinion it should become necessary.

Specific happenings in this connection may tend to emphasize the brief statements which have been made. Some eight years ago or more, the New York City Commissioner of Health, Haven Emerson, called attention to the large number of complaints of incompetent work done by many private laboratories in New York City, and expressed the hope that the New York Academy of Medicine might do something to remedy the condition. In consequence, the Public Health Committee of the Academy made an investigation, and, as a result advised that the Board of Health frame rules and regulations under which private laboratories should be allowed to operate. Beyond the requirements of a license, nothing has been done to date.

About the same time, a similar complaint was made by officers of the American Medical Association to some of the private clinical pathologists of Chicago, with an unofficial request that the laboratory men themselves establish an organization for laboratory standardization and control, which, if suitable and efficient, should win the support of the American Medical Association. An earnest effort in this direction was made under the leadership of Hektoen and others, but the World War prevented the success of this undertaking. It was also about this time that a study of this problem was made by the New York Bureau of Municipal Research, and in an exhaustive report published by this body, the authority of the City Department of Health in the matter was demonstrated under

existing law and compulsory control was strongly advocated in the interest of public health. Hospital surveys under the auspices of the American College of Surgeons, and the activities of the Council of Medical Education and Hospitals of the American Medical Association have given increasing attention to laboratory personnel and equipment.

As it was impossible for all the members of your Committee to meet to consider the problems under discussion, some of the written comments of the absentees may be of interest here.

"Pathologists are often connected with hospitals or private laboratories performing no other function than to give a false sense of responsibility to these laboratories."

"It is most desirable that medical laboratories have physicians who have mastered their specialty in direct charge of the work."

Enough has been said to prove that the danger of existing conditions is generally recognized and therefore it scarcely seems necessary to review in detail evidence of its actual existence. There is no one active in clinical pathology who cannot detail a number of laboratories conducted by insufficiently trained technicians, and even by medical graduates who have no knowledge of laboratory technic, and employ incompetent technicians to do the actual laboratory work. It is also not uncommon to find hospital laboratories equally inefficient for similar reasons, to say nothing of the instances when a competent clinical pathologist through stress of private work exerts no control over his inefficient institution laboratory.

Admitting existing conditions, what can our Association do to remedy these evils, and what will happen if the issue is avoided? These are important questions with professional and economic significance which confront the qualified physician who specializes in clinical pathology and it would seem urgent for our Association to assume as one of its legitimate functions the duty of at least attempting a solution. A careful survey of the situation is urged and a critical examination of plans for relief should become a duty. While self-appointed censorship is on the whole an undesirable method, still if no other scheme is available, it may be necessary to come to it.

For the purpose of opening a discussion which may lead to constructive action by the Association, it is suggested that a Commission be appointed to formulate a method by which the laboratories of Members of the Association may on request secure what may be known as a Certificate of Approval of the American Association of Clinical Pathologists. This method might include qualifications of personnel, standardization of laboratory procedure possibly controlled by inspection, periodic report on submitted specimens or by such method as the Commission may think wise. The details of such requirements and standards might be relatively simple at first, to be improved and perfected as the plan develops and its need and desirability become more evident. At all events, this official approval should be of such high standard as to secure at sight the unqualified endorsement of such bodies as the American College of Surgeons, the American Medical Association and similar organizations.

This procedure, to be sure, will not assure the elimination of the inefficient laboratory because it will not succeed in making the careless or incompetent physician or the lay public pay proper attention to securing efficient laboratory service. It will, however, convince the thinking medical men of the country and the leading organizations of which they are members, that a laboratory standard has been established which they cannot ignore.

We live in an era of profuse legislative activity in the control of almost every human endeavor, and if this should extend to a consideration of laboratories, aside from such activity as a purely medical professional function, as it well may, the existence of an established standard endorsed by leading medical organizations, might easily shape a proper course of action. In the absence of such organization, however, such effort might easily grow to bizarre proportions, of which we have so many examples in other fields at the present time.

The only existing practical standardization and control of public health laboratories is that of the New York State Department of Health, and mention of it has purposely been delayed until now as it may best serve here by way of an example of what can be

done. This plan was conceived and elaborated by A. B. Wadsworth, Director of the Laboratories of the New York State Department of Health, and carried out by and under the supervision of Ruth Gilbert, head of the Diagnostic Laboratory of the Department.

The plan consists of the annual approval of any public health laboratory in the State for specific diagnostic laboratory procedures if efficient personnel, suitable equipment and agreements relative to methods used meet with the standard established by the Department. These requirements are met by details of personnel and equipment stated on the application blank, a series of agreements relative to methods signed by the applicant, inspection by a representative of the Department, and the occasional submission of specimens for examination which are reported to the Department. Samples of the blanks, agreements and certificates are submitted herewith.

This system is of decided value to the Department in estimating the reliability of the laboratories throughout the State and their value in safeguarding public health. It is in the main characterized by courteous cooperation and constructive criticism in which way it renders a valuable service to the laboratory itself, and its existence, without any doubt, urges higher laboratory standards

A similar system of voluntary submission to laboratory control exerted by this Association might be the means of convincing the thinking members of the medical profession that an absolute laboratory standard has been established. It will be of little or no use unless a very large proportion of the truly efficient laboratories of the country join in the movement, and they will not do so unless the control is exercised by most competent authority in a tactful, courteous and constructive manner.

The object of this report is to lay the matter before you for discussion, and if you see virtue in it as we do, to recommend the appointment of a commission to work out the many essential details and to present to you a complete working plan.

DISCUSSION

President MacCarty.—I sincerely hope we have all paid close attention to this report. I know how seriously the Committee has taken this problem, and I know that it has a very constructive, charitable and tolerant plan. The members of the committee mean to carry out the reform they desire by education rather than by legislation, and I think that that was quite evident in Sonden's report. I hope you will all think about this very seriously, and discuss it before our business meeting on Saturday, because I think we should do something with this very excellent report.

Dr. L. A. TURLEY, Oklahoma City, Okla., read a paper entitled **Spinous Cell Carcinoma.** (For original article, see page 272.)

DISCUSSION

Dr. Turley.—I want to say, personally, that I have experienced some disappointment in the discussion of the papers up to this time, and I hope there will be some discussion of my paper. This morning I heard a man say, "I would have discussed a certain paper yesterday, but I did not agree with the author." I hope no one will hesitate to discuss this paper, because of the fact that he does not agree with me. I can always learn something from a man who disagrees with me, and that is one of my purposes in life—to learn as much as I can. (Paper read)

Dr. Hillkowitz.—I do not think the appeal of Turley for a discussion of his paper should be left unheeded, if for no other reason than disagreeing with him, which I am constrained to do, particularly in view of the fact that our President this morning told us that there are 1138 different pathologic entities in our nomenclature, and today one more I think would be like the proverbial straw that would break our back. Turley's nomenclature would be acceptable if it would add anything from the standpoint of helping the pathologist, either in diagnosis or prognosis. As MacCarty pointed out, the average clinician wants to know whether the tumor is benign or malignant, or how benign or malignant it is. Now it seems like splitting hairs merely to state that the squamous cell is not, strictly speak-

ing, a squamous cell, but has its various dimensions and three points in space. So far as the prognosis of the patient is concerned, we are aware that the squamous cell variety is more apt to produce metastasis, but to inject a new classification of spinous cell and say categorically that certain tumors arise from the spinous cells, thus overthrowing, if I understand it rightly, our previous conception that there is a transition from the lower layers, gradually growing into the spinous cells, and then into the dead cells at the top; such a classification is a revolutionary step which I am not yet willing to accept. Nevertheless, you will pardon me, if I express the usual compliments of gratitude to Dr. Turley for bringing this information to our attention. It is a subject well worthy of discussion and consideration.

Dr. Turley (in closing).—I am very glad that the Doctor's discussion has brought these points out. So far as the new term is concerned, and MacCarty's statement that there are now listed 1138 different kinds of tumors, I heartily agree with both men that we should simplify the nomenclature and reduce the number of tumors if possible. I recently received a letter from an eminent pathologist and clinician in this country, who has a man working under him, who has made out, I think, something like twelve different kinds of tumors that are, all of them, when you come to examine them, this same spinous cell carcinoma. This I should say was superfluous addition of names and terms. I think we should have a simple classification and that we should stick to it; but I think the point is also true, if the pathologists are going to talk about the same thing, that we should have the same terms so that we understand exactly what they mean. For instance, when a patient comes from one part of the country to another with a diagnosis of a certain type of carcinoma, not only the one who made the diagnosis but the one who reviews the case will know just what the diagnosis means. Let us say he has an epidermoid carcinoma, which as the Doctor pointed out, has a certain type of malignancy; now if that includes a whole variety of tumors, for example, all possible tumors that might develop from stratified squamous epithelium the diagnosis means as little as the term "bilious." It is as misleading to the surgeon and clinician to restrict our number of kinds, if they are essentially different, as it is to have too many kinds, because different varieties of tumors have different habits of malignancy and growth. A definition should be inclusive and exclusive; that is, one should include all of a certain variety and exclude all the other varieties. In referring to the second point that Hillkowitz brought out, I would like to ask him if we should keep all old theories simply because they are old, or because it is going to disturb our equanimity by requiring us to learn something new? If it can be shown that the old theory is erroneous, why not change to a correct one?

DR. E. H. RUEDIGER, Los Angeles, California, read a paper entitled **Quantitative Wassermann Test**. (For original article, see page 303.)

DISCUSSION

Dr. John A. Kolmer.—I thoroughly agree with the principle of using a quantitative Wassermann reaction, because of a very important biologic reason. If one conducts the test with a constant or fixed dose of serum, as one-tenth or two-tenths of a c.c., we may occasionally observe that with these amounts of serum that the reaction is weak, or, indeed, might be negative with the serum of a syphilitic. With my own method, I think this happens about once or twice in each one hundred sera, so that from the standpoint of accuracy there is probably this very important reason for using the serum in at least two or three doses. I am inclined to think that the phenomenon can be observed with practically any method in common use at the present time, irrespective of the technic being employed; I believe it is well for those who are using one dose of serum with their present method to modify it slightly in order to carry one or two extra tubes with a smaller amount of serum. We have not noticed this so far with spinal fluids and I do not know why the phenomenon occurs. Naturally, of course, one first thinks of the presence of antisheep hemolysin. Our own experience, however, shows that while this might be one factor, it is not the whole story. It might be, as I think Sanford has suggested, a phenomenon due

to the presence of salts in the plasma. We do not know just the mechanism concerned, but I think it constitutes a very important biologic reason for conducting our test, irrespective of the method we are employing, with at least two or three doses of the serum, in order to obtain a greater degree of accuracy insofar as positive and negative is concerned. Another, much less important reason, is to give a graphic record of serologic improvement in the treatment of syphilis. I think this is quite important, but much less important than the first reason; but often, physicians who are engaged in the treatment of syphilis, after noting considerable clinical improvement in the patient, are rather surprised that the serologic reaction is without improvement. With the old method, the reactions continue to be plus-4, and if the physician acquaints his patient with this result, it can be very depressing indeed. As a matter of fact, I think those of us here who are engaged in the treatment of syphilis have learned not to acquaint our patients always with the results of the Wassermann test, because it is frequently very depressing. However, when a quantitative reaction is conducted, we can usually show by using varying amounts of serum, a greater serologic improvement, and this is quite important to the physician and his patient. This constitutes the important reason for conducting a quantitative method, irrespective of the method that any of us may be using.

Dr. A. H. Sanford.—I heartily second everything Kolmer has said. About two years ago, it seemed to us rather formidable to attempt to do quantitative Wassermanns, doing as many as we do every day, but since 1922 we have done quantitative Wassermanns on spinal fluids, and on serum, when it is requested, to the full five-tube quantity, and in two-tube quantities in all the tests. We are far more satisfied ourselves and we know that the dermatologist is far more satisfied.

Dr. Marquez.—My experience with the Wassermann reaction is rather unsatisfactory. Whether that is due to my technic, or due to the imperfect clinical reports that we receive in the laboratory, I do not know. I have followed Kolmer's technic to determine the Wassermann reaction, and I have come in contact with clinicians who criticize his reaction, for the simple reason that in two instances when my curve came down toward the negative line in blood sera, the patient was developing symptoms of tabes, and they could not understand why this was so. We laboratory men can readily appreciate that; but our fight is with the practicing physicians. If we could only convince them that this quantitative reaction of Wassermann is of value, in spite of its occasional failures, we would accomplish very much toward the standardization of our quantitative Wassermann test. It is to be admitted that in time we shall have the standardized Wassermann reaction, and the physicians will appreciate and read it with just as much understanding as we do, so we must not get impatient with our brother practicing clinicians.

Dr. Ruediger.—This phenomenon of a negative result with human serum of low dilution and a positive result with the human serum in a higher dilution is not entirely due to the normal antisheep hemolytic amboceptor in the human serum, because it is also seen when the antihuman hemolytic system is used in which case there is no such excess of hemolytic amboceptor. I cannot explain the cause; about four years ago, in a report on this subject, I showed that it completely disappeared with aging of the serum. I preserved the human serum, tested it from time to time and found that it usually disappeared in less than a month. So far as the attitude of the clinician toward the quantitative Wassermann test is concerned, we sometimes meet opposition from those unfamiliar with it. Those who are not used to quantitative reports can usually be persuaded to accept them; but after they have become used to quantitative reports they usually cannot be persuaded to go back to the qualitative reports.

til the advent of prohibition it had been a tradition among the employees to get as much beer as possible into the stomach when one of their fellow workers had been gassed. During recent years ginger ale has been substituted. Carbon dioxid appears to be absorbed from the stomach. It is absorbed with quite surprising rapidity from the peritoneal cavity.

Patients treated by the Henderson-Haggard method inhale roughly one liter of carbon dioxid at atmospheric pressure per minute. It is of course uncertain whether the volume of carbon dioxid absorbed from beer or ginger ale in the amounts that can be retained by the stomach, would be sufficient for pronounced stimulative effect upon the respiratory center. But there can be no gainsaying the fact that the administration of beer and ginger ale appeared to be beneficial in a sufficient number of cases, probably chiefly mild and early ones, so that this emergency relief measure had become traditional among the employees of the gas plant.

<div align="right">—W. T. V.</div>

The Hospital Laboratory and Research

IF one may judge by hospital reports, the activity and efficiency of the hospital laboratory is most frequently indicated by the number of examinations made during the year; but a far more inclusive and reliable criterion, affording a more accurate estimation of the true efficiency of the laboratory, as well as indicating in a decisive manner its true place in the hospital's activities, is to note the degree to which it initiates, seizes upon, and develops opportunities for laboratory and clinical research and investigation. The index to these activities is best indicated by the publications issued from the laboratory during the period covered by the hospital report.

It would seem that there is a tendency, especially in the smaller hospitals, to look upon research as connoting, far-reaching problems of vast magnitude possible only to institutions especially equipped for the purpose. This thought, however, is fallacious and leads, too often, to lethargy, inertia, and neglect of the opportunities and material at hand and clamoring for recognition.

There is no greater problem nor any of more import than the study of disease and the means available for its recognition and treatment. New methods of laboratory investigation and modifications of old ones are continually being advanced. These must be evaluated by comparative trial and their significance determined.

The study of disease and the development of its mechanism and manifestations is the study and recording of minutia. There is no hospital the thoughtful consideration of whose laboratory records, if carefully kept and representing careful and consistently thorough work, cannot be made profitable—if not to medicine as a whole, then to the personnel of the hospital itself. There are few, indeed, in which some studies cannot be carried on and which cannot—as they should—contribute their mite to medical knowledge and progress.

Even in those where the work, perhaps because of lack of enthusiasm or initiative on the part of the director, or lack of cooperation on the part of the clinical staff, has assumed a routine, hack character, if records are kept and studied something must come of it; either such and such a routine examination is of no real value and should be discarded, or it is informative or could be made so—in either case an addition has been made to the sum of existing information.

Every hospital with a maternity and, especially, an obstetrical dispensary, however small, is a potential source of information as to the significance of the various blood-chemistry findings in pregnancy and its complications; the medical wards furnish unlimited opportunities for the accumulation of similar records to be later studied and analyzed; the dispensaries and the surgical wards are teeming with suggestive opportunities. No extraordinary vision nor the inspirations of genius are required or demanded—all that is necessary is enthusiasm and the impetus toward and the capacity for work.

Serology is bristling with queries to be answered. Methods are proposed whose value can only be determined by analytic and critical trial; the value and significance of complement-fixation tests upon the cord blood is still a matter of dispute; the relative value and specificity of various antigens and technical modifications is open to study—and so on *ad infinitum.*

Even the records of the humble urinalysis may be scrutinized with profit, compared and correlated, perhaps, with the blood chemistry. Better results and better methods of reporting them are always within the realm of possibility. The enthusiasm of even one worker may serve as the means of awakening that of others. It is not sufficient to keep laboratory records—they should be studied and without fail will furnish plenty of food for thought and queries for elucidation Even if nothing but the crystallization and clarification of one's own knowledge is accomplished, that in itself is something and often leads to further problems and activities.

It should be demanded and expected of every hospital laboratory and of its director that at least one paper embodying some investigative activities should come from it each year—nor should less than this be demanded of the clinical divisions.

When one sees a hospital, perhaps of some size, as one may—with an imposing list of admissions; with laboratories well equipped as to apparatus and personnel, and observes that the hospital report carries merely a tabulated list of the number and kind of laboratory examinations made and reports rendered, and fails to find in the index of current literature any mention of reported observations or investigations begun—there are only two possible explanations which come to mind: Either the knowledge and information gained from its work is closely and selfishly hoarded within the institution; or else its work and its personnel are fast sinking into a state of lethargy and innocuous desuetude undisturbed by any quiver of that acquisitive spirit of inquisitive scientific curiosity which leads to the endeavor, at least, to extend one's knowledge.

—*R. A. K.*

Infections Due to the Fusiform Bacilli and Spirochetes of Vincent

THE acute inflammatory condition first described by Vincent associated with the presence of fusiform bacilli and spirochetes in the lesions has, until rather recently, been regarded as a relatively infrequent and comparatively trivial infection.

Its occurrence, however, during the World War in extensive and even epidemic form as an ulcerative gingivitis in large bodies of troops has awakened and renewed interest in this condition and its causative organisms.

Prior to the experiences met with during the war this infection was generally associated mentally, particularly with infections of the tonsillar and pharyngeal areas; that it could be responsible for lesions limited to the gums was, perhaps, not generally appreciated.

The condition has always been of some interest bacteriologically because of the apparent indissoluble symbiotic relationship existing between the bacillus fusiformis and the spirochetes with which it was always found associated.

It was, apparently, not possible to grow these organisms separately in pure cultures in spite of repeated attempts by various workers.

This failure and this apparent symbiosis are believed by Tunnicliff[1] to be due to the fact that the organisms are really one and the same, the spirochetes being but a stage in the life cycle of the B. fusiformis. While this conclusion has been disputed by other workers and Tunnicliff's observations have not yet been confirmed or accepted by bacteriologists at large, the evidence presented which is based upon the study of pure cultures, cannot be lightly cast aside.

Partly because of isolated observations from which somewhat unwarranted conclusions have been drawn; because of the fact that syphilitics, are, apparently, somewhat susceptible to this infection; and because of the further fact that arsphenamine and its derivatives are extremely effectual in the treatment of the disease, being practically a specific, there has been—and especially among the dental profession—some tendency to consider the condition as related to or associated with syphilis in some way.

Thompson,[2] for example, is quoted as authority for the statement that a positive complement-fixation reaction occurs in Vincent's angina in the absence of syphilis; and this impression, which it is somewhat difficult to trace to its source, has gained some circulation. These findings, however, have not been corroborated by observers working with modern and perfected methods, and even failed of substantiation as long ago as 1918 by Taylor and McKinstry[3] whose studies were specifically directed toward this point.

Soberheim[4] has been often referred to with reference to the supposed relation between syphilis and Vincent's angina. His evidence, however, consists of three cases, two of which were very evidently concurrent infections, and in the third syphilis could not be ruled out.

Vincent's angina does occur with some frequency in syphilitics and especially in those whose dental hygienic conditions and individual reactions to specific therapy lead to the occurrence of stomatitis; whether or not it occurs more frequently in the syphilitic than in the nonsyphilitic, statistics are not available to indicate and the question is of relatively little significance other

than as indicating the favorable preparation for secondary invasions by the inflammatory reactions accompanying or initiating stomatitis due to excessive drug reactions.

By some observers the organisms of Vincent's angina are looked upon as essentially secondary invaders; opposed to this view, however, are the facts that: the organisms are found only infrequently in the clean and healthy mouth; they are common in infectious conditions of the teeth, gums, and tonsils; the disease may occur in epidemic form and it responds rapidly to neoarsphenamine with a simultaneous disappearance of both the lesions and the organisms.

There is, of course, as little reason for assuming a relation between the spirochetes of Vincent and the Spirocheta pallidum because both are destroyed by arsphenamine as for assuming a relation between the typhoid bacillus and the Staphylococcus because both are killed by bichloride of mercury solutions.

If gingivitis due to the organisms of Vincent occurs with relative frequency in syphilitics it furnishes simply another potent argument for the necessity for strict and thorough dental hygiene in syphilis.

Fortunately, arsphenamine and its derivatives furnish a practically specific method of treatment as ample evidence proves Barenberg and Bloomberg[5] recently report brilliant results with sulpharsphenamine either locally or intramuscularly. While treatment has been given intravenously as well as intramuscularly, equally good results have been obtained by local applications of the drugs in suspension in glycerin.

It seems possible that the organisms responsible for this infection may be even more widespread than has heretofore been thought. Brams and Pilot[6] in a study of erosive balanitis, which condition, as is well known, is not uncommonly associated with, and may be due to the presence of fusiform bacilli and spirochetes, were led to make examinations by smear and culture of the preputial secretion of one hundred normal men with the demonstration of these organisms in 51 per cent Similar examinations in women gave an incidence of 58 per cent of positive findings.

The frequent presence of these organisms in the teeth and tonsillar crypts has been noted by Davis and Pilot[7] and many others.

In view of these facts and of the extensively destructive lesions which these organisms are capable of producing, it is fortunate that specific as well as rapidly effectual methods of treatment are at hand.

REFERENCES

[1]Tunnicliff, R.: Life Cycle of B. fusiformis, Jour. Infect. Dis., August 1923, ii, 147.
[2]Thompson, St. Clair: Dis. of the Nose and Throat, Cassell, London, 1916, Ed 2, p. 415.
[3]Taylor, F. G. and McKinstry, W. H.: A Serologic Investigation of Vincent's Angina, Jour. Laryngol., Rhinol. and Otol., London, 1918, xxiii, 289.
[4]Soberheim: Arch. f. Laryngol. u. Rhinol., 1909, xxi, 5.
[5]Barenberg, L. H. and Bloomberg, M. W.: Use of Sulpharsphenamine in Vincent's Angina and Stomatitis in Children, Jour. Amer. Med. Assn., July 5, 1924, lxxxiii, 25.
[6]Brams, J. and Pilot, I.: A Study of Erosive Balanitis etc., Arch. Dermat. and Syph., April, 1923, vii, 1.
[7]Davis, D. J. and Pilot, I.: Studies of B. Fusiformis and Vincent's Spirochetes, Jour. Amer. Med. Assn., September 16, 1922, lxxix, 944.

—R. A. K.

BOOK REVIEWS

Medical Quotations[1]

HAVE you not often planned on card indexing remarks, phrases or paragraphs which you have come across in your general reading, bearing upon some subject of particular interest, be it religion, poetry, philosophy or science? This Dr. Lindsey has done in collecting random remarks on medical subjects by various English authors. These include Sir John Mandeville, Sir Thomas More, Francis Bacon. Robert Burton, Sir Thomas Browne, Samuel Pepys. Jonathan Swift, George Berkeley and Laurence Sterne.

Taken at random many have little significance. The quotations of Samuel Peyps, however. which comprise about two-thirds of the volume, are interesting and while they again are unrelated excerpts. they make thoroughly delightful reading.

International Clinics,[2] June, 1924

THE second volume of this series of International Clinics contains interesting articles on diverse subjects. The reviewer mentions particularly, one by Dorland on the influence of x-rays on bacteria, plants, protozoa, organic substances, butterflies, beetles and other insects, intestinal worms, marine forms of life, birds, reptiles and mammals. The experimental work done on normal tissues and on pathologic tissues is reviewed critically. The author emphasizes the dangers to the fetus in radiation of the pregnant mother either for diagnostic or for therapeutic purposes. A comprehensive bibliography is appended.

Balfour and Flynn summarize recent advances in surgery. To a great extent a compilation of recent work at the Mayo Clinic, its scope, is by no means thus delimited. While naturally of interest to the surgeon, the discussion will be of particular value to those interested in others of the medical specialties. in that all unnecessary detail has been omitted, and one may in a few short pages become reasonably well acquainted in a general way with recent surgical advance.

A contribution of distinct promise is that of Graham and Cole referred to by Balfour and Flynn on the study of gall-bladder disease by the intravenous injection of substances opaque to the x-ray and which are excreted through the bile.

International Clinics, September, 1924[3]

THIS volume conforms with its name in that we find herein several interesting contributions from European authors. An essay by Sherwood-Dunn on the evolution of the treatment of syphilis is of interest in that it enables us to contrast American methods with the continental routine. The conclusions are naturally quite similar but there appear some noteworthy variations in the standard course of treatment.

Another European contribution on the treatment of the various types of allergic disease serves to remind and to convince us that the real advances of the last few years in the treatment of asthma, hay fever and allied conditions, have been made especially by American investigators.

Hipwell and Gilchrist give a word of hope concerning insulin, in that they report a series of cases in which diminution of insulin dosage in the course of the treatment of diabetes has suggested very strongly the existence of truly curative properties.

Zingher summarizes recent work on scarlet fever, describing particularly the Dick test and the process of immunization. His description is accompanied by color plates

Calorimetry in Medicine[4]

THIS is not a handbook for clinicians. The author has devoted no space to the technic of determination of the basal metabolic rate and has incorporated no tables for computation The work is essentially a monograph on metabolism in health and disease rather than as the name would suggest, a treatise on the procedure of measuring heat production. The author emphasizes that the diagnostic use of basal metabolic rate determinations in dysthyroidism, while of unquestioned value, is probably of less importance than is the information to be gained from a study of heat production and heat regulation in various other pathologic states. He emphasizes that the study of total energy transformations in disease should no longer be confined to the postabsorptive state but that the effect of the ingestion of food and the cost of muscular work should receive further investigation.

The book is essentially a résumé and discussion of the valuable work of all investigators, including that of the author, on the basal metabolism in diseases such as pregnancy, nutritive disturbances, fever, diseases of the circulatory system, etc. The effect of drugs and particularly of the various foods on the basal metabolism is discussed.

The work should serve as a valuable reference guide in the clinical interpretation of the results of metabolism studies.

[3]International Clinics. Vol. iii September, 1924.
[4]Calorimetry in Medicine. By William S McCann. Associate Physician, Johns Hopkins Hospital. Cloth. Pp 98 Price $2.25 Williams & Wilkins Co , Baltimore, 1924.

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The Next Annual Meeting Will Be Held in Philadelphia May 21-23, 1925
Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 21, 22 and 23, 1925, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering next year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.

CLINICAL AND EXPERIMENTAL

THE RELATION OF THE CHLORIDE METABOLISM TO THE TOXEMIA OF LOBAR PNEUMONIA*

Preliminary Report

By Russell L. Haden, M.D., Kansas City, Kansas

LOBAR pneumonia is a complex disease in which any one of several factors may play the leading rôle. In some cases a bacteriemia may be the fatal feature, in others the deficient oxygenation may seem the most important factor. Quite typically, however, there is a toxemia which is responsible for many of the serious symptoms of the disease. The toxemia may perhaps arise indirectly from the bacteria concerned in the infection. It seems quite possible, however, that it may arise as a by-product of the bacterial infection which is the initial feature. This possibility is made more than probable by certain chemical findings which indicate a close relation of the toxemia of lobar pneumonia to other toxemias in which a bacterial factor can be excluded. The most typical chemically related toxemia is that occurring in intestinal obstruction.

The very low chloride excretion in acute lobar pneumonia has long been recognized as one of the most characteristic features of the disease. The behavior of the chlorides and the termination by crisis sharply differentiate lobar pneumonia from other acute pathologic conditions of the lung in which consolidation of tissue occurs. There is usually also increased protein destruction as indicated by the high nitrogen excretion.

In studying the chemical changes in the blood of the dog, following experimental intestinal obstruction, Haden and Orr have found that chlorides disappear rapidly from the blood usually with a coincident alkalosis. These changes precede the tissue destruction and increased nonprotein nitrogen con-

tent of the blood. The toxemia which is the most characteristic feature of high intestinal obstruction, does not arise until the chlorides have fallen below a certain level. Haden and Orr[2] have also shown that by keeping the blood chlorides near the normal level the toxemia may be prevented entirely. Dogs with obstruction of the jejunum have been kept alive as long as thirty-one days without developing a toxemia, while untreated dogs die in four days on an average. Likewise, supplying chlorides after the blood chlorides have reached a low level and the toxemia is already under way has usually influenced, most favorably, the course of the toxemia.

The fact that the toxemia does not develop in untreated animals until the chloride reserve is well depleted and can be prevented by providing an ample supply of chlorides, seems to prove that the utilization of chlorides is a protective mechanism on the part of the body. The changes are possibly explained by supposing that the toxic body arising as a result of the intestinal obstruction is converted by the sodium chloride into a nontoxic body with the formation of sodium bicarbonate as a by-product. Other possible explanations have been discussed elsewhere.[2]

The study reported here has been made with a view of determining, if possible, the relation of the chloride metabolism to the toxemia in lobar pneumonia as reflected in changes in the blood. The observations are rather fragmentary but seem worth while recording. Peabody[3] and others have studied the chlorides of the blood during lobar pneumonia and found them at a low level. It has been shown that the exudate in the consolidated lung contains only a small fraction of the chlorides retained. Analysis of other body tissues has not revealed any significant increase in chlorides. The very low excretion is due to the fact that the chlorides in the blood are below the renal threshold. No satisfactory explanation has been given for the disappearance of the chlorides from the blood. Peabody concludes that the chlorides retained may be generally distributed over the body.

Recent chemical studies on the blood in pneumonia[4] have shown also, that there is frequently a high nonprotein nitrogen in the blood. A large proportion of the nonprotein nitrogen is in the undetermined fraction, indicating that the changes observed are due to increased tissue destruction and not primarily to kidney retention. Studies on the CO_2 combining power of the blood plasma[5, 6, 7] show that there is seldom an acidosis, a condition so characteristic of other infectious diseases. There is often a true alkalosis. The low chloride excretion, the low level of blood chlorides, the frequent alkalosis, the increased protein destruction with the high level of nonprotein nitrogen in the blood in both lobar pneumonia and intestinal obstruction, indicate that there is a close relationship in the chemical changes taking place in the two diseases. As a further evidence of the similarity the occurrence of melanuria in both intestinal obstruction and in lobar pneumonia has been observed.[8]

The very striking results in both preventing and combating the toxemia of intestinal obstruction by simply keeping the blood chlorides near the normal level, suggests its use in the chemically related toxemia of lobar pneumonia. None of the recent articles on the treatment of lobar pneumonia such as those in Nelson's Loose Leaf Medicine or the Oxford System

of Medicine mention the use of sodium chloride. Numerous articles were published twenty to twenty-five years ago on the saline treatment of lobar pneumonia. The first clinical report is that by Penrose in 1899.[9] Solis-Cohen[10] gives the credit for its use originally to F. P. Henry of Philadelphia who, he states, used it as early as 1890

Most clinicians employed saline infusions as a means of producing increased urinary excretion. Others however definitely used sodium chloride as a detoxifying agent. Thus Sajous[11] thought that the early use of saline solution would produce the effects one tries to get with specific immune sera. Wakefield[12] pointed out that chloride is a sort of prototype of oxygen and can enter into combination in place of oxygen in conditions of suboxidation. This observer thought that antitoxins in pneumonia are produced by the catalytic action of oxidation The utilization of chlorine, according to

TABLE I

CHEMICAL FINDINGS IN THE BLOOD OF PATIENTS WITH LOBAR PNEUMONIA

PATIENT	DAY OF DISEASE	BLOOD MG. PER 100 C.C.						URINE		REMARKS
		TOTAL NONPROTEIN NITROGEN	UREA NITROGEN	CREATININE	URIC ACID	CHLORIDES	CO₂ COMBINING POWER	CHLORIDES	NITROGEN	
		mg.	mg.	mg	mg	mg	Vol. per cent	per cent	per cent	
1	3	65.5	32.3	1.8	4.9	380	43.8	0.08	0.56	Crisis pre-
	4	72.8	32.2	1.7	3.4	400	44.7	Faint trace	0.63	ceding night
	5	78.0	41.1	1.5	4.1	400	45.7			
	6	82.5	40.6	1.5	4.0	410	55.1			
	8	57.8	26.2	1.5	3.6	390	47.5	0.23	0.57	
	10	45.6	21.0	1.6	4.1	400	49.4	0.40		
	12	30.3	13.1	1.6	4.0	420	49.4	0.70	0.61	
2	3	30.0	14.0	1.6	4.4	360	47.5	0.10		
	4		15.8	1.7	3.9	350	46.6			
	6		14.0	1.7	3.6	370	49.4			
	8	29.4	12.1	1.8	4.7	360	49.4			
3	11	41.6	19.6	1.4	3.6	390	53.2	0.06		
	12	41.6	17.8	1.4	3.4	350	52.2	0.07		
	13							0.10	0.74	
	14	34.9	18.2	1.4	3.8	410	55.1			
	16	30.0	16.4	1.5	3.4	410	49.4	0.80		
4	6	45.0	16.3	1.5	3.6	400	37.6	Faint trace		
	7	60.0	28.5	1.4	3.6	430	45.7			Crisis
	8	69.0	31.8	1.8	4.6	430	41.9	0.05		
	9	57.8	27.0	1.2	5.3	440	45.7			
	10	63.2	21.4	1.2	4.6	410	43.8	0.05		
	11	54.6	16.4	1.2	4.7	470	45.7			
	12	56.4	16.8	1.2	4.7	460	46.6	0.08		
	13	42.8	14.5	1.2	4.8	470	36.2			
	15	32.6	15.4	1.3	4.7	460	44.7			
5	5	33.3	9.34	1.2	3.4	430	32.8	0.15		
	6	52.6	26.2	1.3	4.4	440	45.7	0.10		
6	7	98.4	50.0	1.8	3.6	390	36.2			

TABLE II

CHLORIDE BALANCE OF PATIENT WITH LOBAR PNEUMONIA TREATED WITH SODIUM CHLORIDE

DATE	SODIUM CHLO-RIDE GIVEN	URINE CHLORIDES		BLOOD	
		PER CENT	TOTAL	CHLORIDES (PER 100 C.C.)	CO$_2$ COMBINING POWER
Dec.	gm.		gm.	mg.	Vol. per cent
21	10	0.04	1.44		
22	16	0.07	1.54	370	
23	24	1.04	1.77	390	
24	24	0.08	2.48		
25	.0	0.15	9.30	370	51.3
26	.0	1.13	6.89	400	
27	.0	0.10	1.50	380	
28	.0	0.30	7.50		
29	.0	0.40	5.60	480	55.1
30	6	0.40	5.60	480	
31	6	0.41	4.10	380	
Jan.					
1	6	0.60	5.04	400	46.6
2	6	0.74	6.60		
3	6	0.87	5.22		
4	6	0.80	6.00		
5	6	0.73	1.18		
6	6	1.18	3.78		
7	6	1.23	8.00		
8	6	1.00	11.50		
9	6	1.12	6.77		
10	6	1.00	7.00		
11	6	1.00	7.00		
Total	152		112.61		

this view, is a protective measure on the part of the body in an attempt to hasten the formation of antibodies. Peabody,[3] in studying the blood chlorides in pneumonia, gave small amounts of salt to note what effect it would have on the blood chlorides. Prigge[13] has reported eleven cases of pneumonia in which a single intravenous injection of 20 to 38 gm. of sodium chloride was given. Every one of the patients showed a rise in blood pressure following the injection and all recovered. Prigge states that all of the chloride was excreted within four days after it was given, and that the chloride metabolism in pneumonia patients differed from normal controls only in the greater length of time required to excrete the chloride injected.

It is a surprising but well proved fact that dogs with high intestinal obstruction die more quickly when given a plentiful supply of distilled water than if untreated.[2] Considering the close chemical relationship of the two conditions it seems a question whether it is wise in lobar pneumonia to force water and other fluids containing very little salt.

The observations reported here are based on the study of seven cases during an acute lobar pneumonia. In four, repeated complete chemical analyses of the blood have been made over a period of several days; in two, only a few analyses were made; and in one, only the chlorides and carbon dioxide combining power were studied. The analyses in six cases are shown in Table I. The results are quite variable. In all, the blood chlorides are below the normal level. In five, the nonprotein nitrogen and urea nitrogen are above the upper limit of normal. Two of the patients died. In the other

three the nonprotein nitrogen and urea nitrogen returned to normal as the blood chlorides reached the normal level. The sixth case showed a low blood chloride without a rise in nitrogen. The urine chlorides were estimated in five cases and found very low in all.

The chloride metabolism of one patient treated with sodium chloride is shown in Table II. This patient was a young robust man admitted to the hospital with an acute appendiceal abscess. A typical lobar pneumonia developed the day following operation. At the outset only the right lower lobe was consolidated. Later the middle and upper lobes were similarly involved. The blood pressure remained normal throughout the illness. One other patient with lobar pneumonia due to hemolytic Streptococcus responded equally well to treatment. This patient developed the lung infection following a cholecystectomy. The chart of the chloride metabolism is of little value, however, since some of the chloride tablets were passed unchanged in the stools.

The patient shown in Table II was given 74 gm. of sodium chloride by mouth in the first four days of treatment besides that taken in with food. During this period the total excretion in the urine was 7.23 gm. and the blood chlorides remained well below the normal level. A total of 152 gm. of salt was given by mouth during the period of observation. For the greater part of this time he was taking a soft diet which included about 5 gm. of salt per twenty-four hours. The total excretion was 112 6 gm. This patient had no acidosis.

DISCUSSION

The cases reported here showed no significant decrease in the carbon dioxide combining power emphasizing again the absence of an acidosis in lobar pneumonia. The creatinine is unchanged and the uric acid is little above the upper limit of normal. The low chlorides and increase in nonprotein nitrogen and urea nitrogen are the significant findings. The undetermined fraction of the nonprotein nitrogen is quite high. These findings suggest that the changes observed are due to increased protein destruction instead of kidney retention. It is most probable that the accelerated protein metabolism is generalized and that the products responsible for the toxemia come from body protein largely independent of direct bacterial action.

The urine chlorides are very low, and the urine nitrogen quite high. The urine chlorides give little clue to the level of chlorides in the blood and body tissues.

The chloride metabolism of the patient treated is very striking. He was given 152 gm. of sodium chloride by mouth in addition to that taken in with food. The latter is estimated as being about 5 gm. per day. Only 112 gm. of the 152 gm. given is accounted for in the urine in twenty-one days. The very large amount of salt which will be absorbed in toxemia with chloride depletion is significant. The findings here are very similar to those in intestinal obstruction. One patient with intestinal obstruction has been observed to absorb 90 gm. of sodium chloride in thirty-six hours when given by hypodermoclysis, and excrete during the same time only 1.8 gm.[14]

The clinical condition of the patient treated was very satisfactory, even when one entire side was consolidated. The blood pressure remained normal. One of the most serious aspects of pneumonia is the low blood pressure. The chlorides must play an important part by osmotic action in maintaining the pressure within the vessels.

Sodium chloride was given in 1 gm. enteric coated tablets (Hynson, Westcott, and Dunning). The administration of sodium chloride in this way is a simple procedure. It is suggested that such use is indicated in all cases of lobar pneumonia in which there is a low level of chloride excretion without an evident kidney lesion.

The results in a group of patients so treated will be reported later.

SUMMARY

The blood chemical analyses of a series of patients with lobar pneumonia are reported. The blood chlorides are low, the nonprotein and urea nitrogen are high; there is no acidosis. The changes observed are probably due to a generalized protein destruction. Supplying the missing chloride seemed to influence markedly the course of the disease. In all cases of lobar pneumonia sufficient chloride should be given to keep the blood chlorides at or near the normal level.

REFERENCES

[1]Haden, R. L., and Orr, T. G.: Jour. Exper. Med., 1923, xxxvii, 365.
[2]Haden, R. L., and Orr, T. G.: Jour. Exper. Med., 1923, xxxvii, 377.
[3]Peabody, Francis W.: Jour. Exper. Med., 1913, xvii, 71.
[4]Killian, John A.: Proc. New York Path. Soc., January-May, 1922, xxii, 72.
[5]Whitney, J. L.: Arch. Int. Med., 1917, xx, 931.
[6]Palmer, W. W.: Jour. Exper. Med., 1917, xxvi, 495.
[7]Barach, A. L., Means, J. H., and Woodwell: Jour. Biol. Chem., 1922, 1, 413.
[8]Haden, R. L., and Orr, T. G.: Bull. Johns Hopkins Hosp., 1924, xxxv, 58.
[9]Penrose, C. A.: Bull. Johns Hopkins Hosp., 1899, x, 127.
[10]Solis-Cohen, S.: Am. Med., 1901, i, 293.
[11]Sajous, C. E. de M.: Internal Secretions, 1905, p. 787, Quoted by Taylor, J. M.: Med. Rec., 1906, 69, 49.
[12]Wakefield, Homer: Med. Rec., New York, 1906, lxix, 49. (Discussion).
[13]Prigge, R.: Deutsch. Arch. f. klin. Med., 1922, cxxxix, 1.
[14]Haden, R. L., and Orr, T. G.: Surg. Gynec. and Obstet., 1923, xxxvii, 365.

STUDIES ON THE PHYSICAL AND COLLOIDAL CHEMISTRY OF ARSPHENAMINE

PART I. THE DETERMINATION OF AN ISOELECTRIC POINT*

BY A. STUART HUNTER AND W. A. PATRICK, BALTIMORE, MD.

(From the Department of Chemistry, Johns Hopkins University.)

ALTHOUGH years have passed since salvarsan was first presented to the world by Ehrlich and his collaborators, but little real information has been acquired as to its chemical action when in solution, the cause of its frequent abnormal toxicity, and the complex problem which its physiologic activity in the body presents. In spite of the vast amount of work that has been done upon this drug, from a chemical as well as a clinical viewpoint, it is impossible to explain the apparent differences which occur in the different commercial arsphenamines on the market today. There is lacking the reason for their variations in color, arsenic content, solubility, toxicity and therapeutic activity.

The application of pure physical or colloidal chemistry to these investigations has thus far been scanty. Bauer (1919) in his study of the colloidal properties of arsenicals of the arseno type measured their diffusibility by means of the ultra filter according to the method of Bechold (1919). He found that the greater part of the salvarsan hydrochloride in solution was retained by the filter, thus showing the presence of colloidal properties. In addition, he noted that a freshly alkalinized solution diffused more slowly than did a solution made from the dry commercial salvarsan sodium.

Sherndal (1921) showed the colloidal nature of arsphenamine very plainly He found that the conversion of the "base" into the dihydrochloride in an ionized medium gave, in every case, a product whose gelatinous characteristics, as evidenced by the viscosity of the aqueous solutions and its insolubility in methyl alcohol, were very much more marked than when the material was prepared in a nonionizing medium. He was able to change a difficultly soluble preparation into a readily soluble one and vice versa.

Raiziss and Gavron (1922) made a qualitative study of the diffusibility of several arseno preparations through parchment membranes. Both the disodium salts and the dihydrochlorides possessed colloidal properties as shown by their partial retention. The sodium salt of arsphenamine diffused to a greater extent than the dihydrochloride.

Z. Klemensiewiez (1920) studied the viscosity of aqueous salvarsan solutions. He found that upon standing, the viscosity of the solution arose to a

maximum, the rate being proportional to the concentration. He classified aqueous "606" solutions as emulsoids together with gelatine, albumin, etc. He suggested the idea that solutions of salvarsan give rise to strong polymerization of the molecule, probably accompanied by hydration.

Jean Oliver (1923) stated that arsphenamine combined with the red blood cells and, in the presence of electrolytes, coagulation resulted. This coagulation, he claimed, was inhibited by the addition of hydrophylic colloids, such as gelatine, gum arabic, starch, etc., which by their combination with the arsphenamine prevented its union with the blood cells and the electrolyte, thereby preventing coagulation. The colloid played the rôle of a protective colloid.

In contrast, however, Freundlich, Stern and Zocher (1923) stated that in the presence of gelatine, arsphenamine was sensitized toward the coagulating effect of electrolytes. In hydrochloride solutions as well as in the alkalinized solutions a .4 molal concentration of NaCl caused marked clouding. Freundlich remarked that this fact should be emphasized. "It should not be overlooked that the hydrophyl solutions so often designated as protective colloids, as gelatine for example, very often exert a sensitizing effect which is exactly opposite. We would put the solutions of salvarsan and neosalvarsan in the class of semicolloids, particularly like the colloidal electrolytes, of which class soap solutions are more familiar. As contrasted with these there is present in salvarsan a colloidal cation but no colloidal anion as in neosalvarsan and the other materials mentioned."

Voegtlin, Johnson and Dyer (1924) in their work on the viscosity and toxicity of arsphenamine solutions showed that a given viscosity of a hydrochloride solution apparently corresponded to a definite toxicity of the product, regardless of the method of manufacture. Also, the higher the viscosity of the hydrochloride, the higher its toxicity. Yet, the hydrochloride solutions which had the highest toxicity showed the lowest toxicity when converted into the sodium salt.

It now remains to establish something definite regarding the physical state and the colloidal properties of this drug. From its accepted chemical structure as well as from its salt-forming properties it is obviously amphoteric, i.e., it can act either as an acid or as a base. The important thing in the study of an amphoteric material is the position of its isoelectric point.

The conception of an isoelectric point as applied to proteins was due to W. B. Hardy (1900) when he noticed that egg albumen when put into an electrical field migrated in an opposite direction according to whether the reaction of the fluid was acid or alkaline. When the solution was neutral cataphoresis was negligible and under such a condition the particles must be isoelectric. In 1905, Hardy in his paper on "Colloidal Solution" recognized the amphoteric nature of proteins and stated that they could react with either an acid or a base. When the methods of measurement of the hydrogen-ion concentration were developed by H. Friedenthal and Sorensen it became possible to determine the isoelectric point of such ampholytes. Michaelis in 1910 used the method of Hardy to study the migration of albumin particles in an electrical field and according to him, the isoelectric point is that hydrogen-ion

concentration at which the particles migrate neither to the anode or the cathode. Loeb (1919) showed that ash-free proteins could be prepared by washing the protein at its isoelectric point, since there it combined with a minimum of anions or cations. His procedure was to determine the P_H of a protein solution potentiometrically and then add acid or alkali sufficient to bring it to its isoelectric point.

Other methods have been developed for the determination of isoelectric points which are more convenient than cataphoresis measurements. They are based on the fact that at the isoelectric point the osmotic pressure, the viscosity, alcohol required for precipitation, the conductivity, the swelling are at a minimum. Levene and Simms (1923) in their paper, "Calculation of Isoelectric Points" conceive of the isoelectric point of an amphoteric substance as that hydrogen-ion concentration at which it is ionized equally as an acid and as a base.

They show that the usual formula for single ampholytes:

$$\text{I.P.} = \sqrt{\frac{K_a}{K_b} K_w}$$

may be used to calculate the approximate isoelectric point of polyacidic, polybasic ampholytes by using the K_a and K_b of the strongest acid and strongest basic group.

Now, if it should be desired to study the colloidal properties of arsphenamine it would be necessary to first obtain the material free of electrolytes or else the experimental results would mean little. Chemically pure arsphenamine has never been made. If a protein can be purified by washing at its isoelectric point a similar treatment might be effective with arsphenamine. It was deemed fundamental then to determine the isoelectric point so that there might be a foundation upon which future physical investigation might rest.

Conductivity measurements on arsphenamine solutions seemed to offer the most applicable method of attack. It was recognized that due to the partial insolubility of the drug at certain hydrogen-ion concentrations and because of the possibility of polymerization and hydration, that a minimum conductivity measurement might or might not be the exact criteria of the position of the isoelectric point. However, it was hoped that conductivity measurements might shed some light upon the physical condition of arsphenamine in solution. The purpose of this investigation, then, was to establish the isoelectric point of arsphenamine as indicated by a minimum conductivity of the solution and to determine the alkalinization curve of arsphenamine in terms of P_H values.

METHODS EMPLOYED

Conductivity Measurements.—The new feature of the conductivity system was the source of alternating current. A radiotron audion bulb was used to generate pure sign wave alternating current. The authors are indebted to Geo. C. Crom of the Engineering Dept. of the DeForest Radio Telephone and Telegraph Co., through Dr. DeForest, for the following set-up which is not only

simple in its construction but also decidedly effective for this sort of work. (See diagram.)

The three "Duo-Lateral" honeycomb coils of the indicated number of turns were seated in a regular frame. The three condensers had a capacity of 0.1, 0.1 and 1.0 microfarads respectively. A radiotron tube U.V. 201 was used. The entire circuit was housed in a cabinet according to the most improved radio methods. Ninety volts were impressed on the plate by the "B" battery and a six volt storage battery served as the "A" battery.

Not enough can be said of the efficiency of this instrument as a generator of alternating current, (DeForest 1920), for conductivity work. The aggravating hum of a buzzer is absent. The cost of a Vreeland oscillator often makes its use prohibitive. This instrument is relatively quiet, enough so that it can be kept within easy reach of the rest of the apparatus and the constant temperature bath. Simply by turning the knob of the rheostat which controls the current passing through the filament, a smooth, clear tone is heard in the phones. This can be regulated at will to any intensity required by the operator. In addition, the current flows through the cell for a minimum length
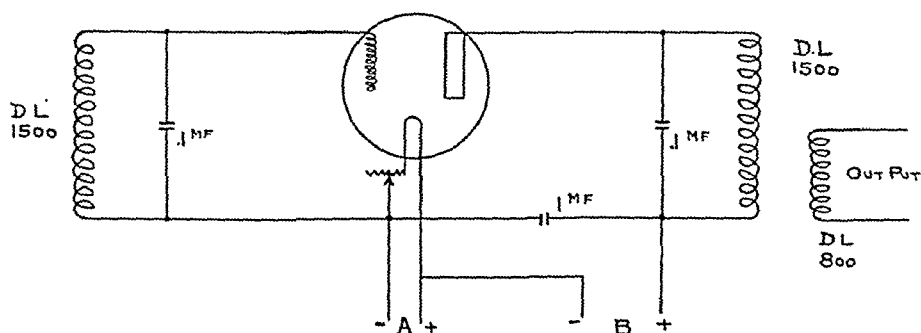
Diagram of Vacuum Tube as a Generator of Alternating Current.

of time and this of course is essential with arsenic-containing solutions, lest the platinum be poisoned. If the bridge drum is perfectly clean and preferably when the wire is covered with a slight film of oil, an *absolute* and *reproducible* null point can be readily obtained between two or three graduations on the drum scale. It was not necessary to have the current passing through the cell for more than half a minute at a time in order to make a measurement and by way of precaution the current was always shut off before the readings were recorded. Three different readings were taken and their mean calculated value was accepted as the conductance of the solution.

The cells were calibrated according to the weight method of Kraus and Parker (1922) using 0.1 N KCl as the standard. The conductivity water which was used in all solutions, was made by redistilling freshly distilled water from alkaline permanganate and calcium hydroxide through a block tin condenser and collecting in Jena glass bottles. When used, the water protected by soda lime, was withdrawn through a Pyrex siphon which had a ground-in stopper at the lower end.

Special cells were made of Pyrex in which the platinum disks 1.0 cm. in diameter were in a perpendicular position about 2.0 cm. apart. A small tube

passed down through the ground-in glass stopper so that an inert gas could be passed over the solutions in order to prevent atmospheric oxidation. An exit tube was placed near the top of the stopper.

The cell constants were calculated according to the usual formula:

$$K = C \times R \qquad \begin{array}{l} C = 0.0128988 \text{ for } 0.1 \text{ KCl } (25° \text{ C.}) \\ R = \text{resistance of solution.} \end{array}$$

The cell constants calculated out as follows:

Cell	C.	R.	K.
A.	0.0128988	14.043	0.181137
B.	"	19.069	0.245950
C.	"	14.925	0.192500

Parallel cells were used for each measurement and the mean of their calculated specific conductivities was taken as the value for the solution. In each series a third cell was brought into use on one or more solutions in order to check the accuracy of the parallel cells and to be sure that they were functioning correctly.

When this investigation was undertaken it was feared that, due to the presence of the arsenic in the solutions, the platinum electrodes might be poisoned and caused to hinder correct measurements. Although some trouble did occur it was only temporary. Great care had been taken that the current flow through the cells for a minimum length of time. Also, when not in use the cells were kept filled with conductivity water. In spite of this there seemed to be a slight absorption on the platinum black as shown by the yellowness of the water in the cells after they had been in constant use for a day or so. The first indications of trouble came after the cells had been in use for about four months. There was an irregularity of sound in the phones. A slight "putt, putt," could be heard and often a ringing sound

TABLE I

CONDUCTIVITY MEASUREMENTS ON ARSPHENAMINE E

NO.	C.C. 0.1 N NaOH PER 0.1 GM.	CELL	L.	MEAN L.	DIFFERENCE
149	0.0	A	0.003171		
		C	0.002993	0.003082	0.000178
151	1.0	A	0.002416		
		C	0.002556	0.002486	0.000140
152	2.0	A	0.002201		
		C	0.002169	0.002185	0.000032
153	2.6	A	0.002132		
		C	0.002038	0.002085	0.000094
154	2.8	A	0.002106		
		C	0.001954	0.002030	0.000152
155	3.0	A	0.002159		
		C	0.000159	0.002159	0.000000
157	5.0	A	0.002664		
		C	0.002585	0.002624	0.000079
150	8.5	A	0.004302		
		C	0.004186	0.004244	0.000116

All solutions were made up on the basis of 1.0 gm. sample of arsphenamine made up to 200 c.c. in a graduated flask. Column 2 gives the c.c. 0.1N NaOH added per .1 gm. sample. Column 6 shows the difference between the two cells to be small.

would predominate. The strange thing about it was that after two or three fresh solutions had been used in the cell it would again function correctly. When in their worst condition readings on the drum meant nothing as a null point was found to be practically constant, regardless of the resistance out. No readings were taken under such conditions.

Finally, the now greyish platinum black was dissolved off with hot aqua regia (the solution being disregarded) and the cells were filled with hot acid chromate for a day. Then after a thorough electrolytic cleansing, they were replated and once more behaved in a normal manner. Any ringing sound usually indicated trouble which always resulted in the necessity of having to repeat the measurement.

The accuracy of the conductivity work is best attested by a typical table (Table I).

Hydrogen-ion Concentration Measurements.—Hydrogen-ion concentration measurements were made in parallel on each solution and simultaneous with the conductivity measurements. Clark shaking electrode vessels were used and the electrodes were covered with platinum black just sufficient to conceal the metal surface and make them jet black and glossy. A saturated calomel cell was made up according to the method of Fales and Vosburg (1918) and a saturated KCl chain was used. The apparatus was kept in an air bath at $25 \pm 0.1°$ C. Electrolytic hydrogen was supplied by a small generator employing platinum electrodes and a slight evolution of gas was maintained continuously when the apparatus was not in use. Constant pressure in the two cells was established by the use of a water manometer within the air bath. Barometric pressure corrections were neglected. The readings were made on a Leeds and Northrup type K potentiometer, and the galvanometer possessed a sensitivity of 0.025 microamperes per millimeter.

Calculations for P_H values were made according to the usual formula:

$$P_H = \frac{\text{Obs.E.M.F.} - \text{Cal}^*}{0.0001983\ T}$$

Due to the slowness with which the precipitate containing solutions came to equilibrium with the hydrogen, twenty minutes was taken as the time of shaking for all solutions before making a final reading. A preliminary reading was made on each solution after shaking for 15 minutes and then a fresh aliquot was placed in the vessels and they were shaken for twenty minutes more. Thus the final and accepted reading represents the condition of the solution after a thirty minute interval. This preliminary measurement was found to be very helpful as it indicated whether or not the parallel electrodes were acting together and correctly. An index of the accuracy of the P_H measurements may be found in Table II.

<center>EXPERIMENTAL WORK</center>

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*E. M. F. Cal, because of consistent reading was taken as 0.2478 volts.

TABLE II

P MEASUREMENTS ON ARSPHENAMINE E

EXP.	C.C. 0.1 N NaOH PER 0.1 GM.	TIME OF SHAKING	ELECTRODE A.	ELECTRODE B.	RECORDED VALUE	P_H
149	0.0	15	0.3889	0.3889		
151	1.0	20	0.3892	0.3900	0.3896	2.390
		15	0.3922	0.3924		
152	2.0	20	0.4052	0.4054	0.4053	2.664
		15	0.4167	0.4162		
153	2.6	20	0.4166	0.4166	0.4166	2.855
		15	0.4449	0.4459		
154	2.8	20	0.4456	0.4462	0.4459	3.352
		15	0.4496	0.4497		
155	3.0	20	0.4492	0.4488	0.4490	3.403
		15	0.4547	0.4549		
157	5.0	20	0.4546	0.4544	0.4545	3.500
		15	0.8484	0.8463		
158	7.0	20	0.8421	0.8423	0.8422	10.05
		15	0.9174	0.9164		
150	8.5	20	0.9134	0.9110	0.9122	11.24
		15	0.9321	0.9332		
		20	0.9288	0.9284	0.9286	11.52

Column 3 gives the time of shaking in minutes; column 4, the voltage readings of the parallel hydrogen electrodes; column 5, the equivalent P_H values.

Philadelphia, Pa.; E. R. Squibb and Sons, New Brunswick, N. J.; Mallinckrodt Chemical Works, St. Louis, Mo.; Kober Chemical Co., Inc., Hastings-on-Hudson, N. Y.; and H. A. Metz Laboratories, Inc., New York, N. Y. In addition, two types of laboratory arsphenamine and two types of salvarsan sodium were made by the authors.

Laboratory Preparations. Arsphenamine D.—Sixty gm. moist "base" was dissolved in 360 c.c. absolute methyl alcohol by the addition of 6.5 gm. 100 per cent HCl in methyl alcoholic solution. The resulting dihydrochloride solution was filtered and precipitated from five liters of absolute ether. The precipitated material, after having been collected on a filter in an inert atmosphere was washed with ether and then dried in vacuo over sulphuric acid. At the end of ten days the arsenic content was 31.95 per cent (control experiment).

Arsphenamine E.—Sixty gm. of the same moist "base" as used in arsphenamine D was suspended in ten liters of distilled water and kept covered with an inert atmosphere. A series of filtercones were inserted into the suspension and while the solution was vigorously stirred, suction was applied. As fast as the water was removed distilled water having a P_H of about 4.0 was added until thirty liters of this acidified water had passed through the suspended "base." Following this, ten liters having a P_H of 3.4 were sucked out. The "base" was then filtered off and made into the dihydrochloride as with D. The arsenic content of this preparation after ten days was 32.35 per cent.

The reason for this modification was to ascertain the effect of washing arsphenamine at its isoelectric point. No marked difference was detected in the physical studies made upon the two products. Toxicity tests have not been completed.

The technic used in preparing and handling the solutions is very im-

Plate I - C -.

Arsphenamine - C -

Specific
Conductivity
X 10^5

pH = 11.5

pH = 11.4 Di-sodium
salt.

pH = 2.4
Di-hydrochloride.

pH = 10.9   Mono-sodium salt.

pH = 7.6
Arsphenamine 'base'.

pH = 3.0
Mono-
Hydrochloride

c.c. .1 N NaOH added to .1 gm. sample (20 c.c.)

0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0

600 575 550 525 500 475 450 425 400 375 350 325 300 275 250 225 200 175 150 125 100
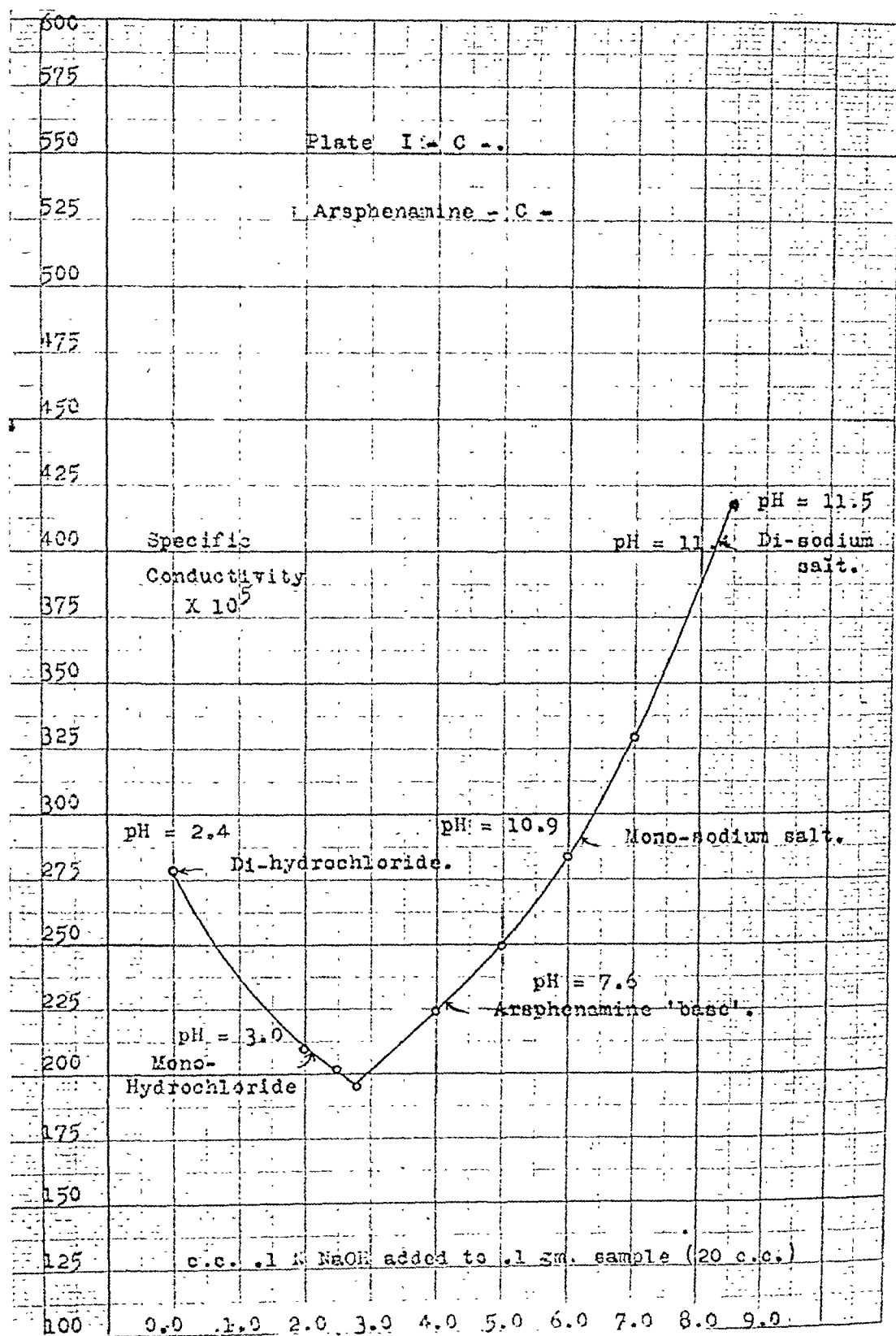
portant as a time factor must be considered. In all cases a uniform concentration of 1 gm. sample made up to 200 c.c. (approximately 0.01 molal) was used. A series of solutions were prepared which contained increasing amounts of sodium hydroxide up to 8.5 c.c. 0.1 N NaOH per 0.1 gm. sample, which corresponds to a correctly alkalinized solution (Public Health Reports, 1922). It is imperative that the sample be completely in solution before the addition of alkali. The alkali was added to the solution from a standard burette and the graduated flask was rotated gently during such an addition in order to prevent undue clumping of any formed precipitate.

When the solution was homogeneous the hydrogen electrode vessels were filled. Following this, some of the solution was placed in the conductivity cells and the time was noted. At the end of fifteen minutes the preliminary potentiometric reading was taken and a second aliquot placed in the vessels. When twenty minutes had passed, the conductivity measurements were made, the cells rinsed out and filled with conductivity water. Then at the end of the second period the final P_H reading was taken. The best proof of the uniformity of this technic came in the fact that the points shown on the curves were not made in consecutive order but that it was possible to reproduce the measurements and insert unknown points in the curves at any time and have them fall within experimental error. All measurements were made at 25° C.

TABLE V

ARSPHENAMINE C

EXP.	C.C. 0.1 N NaOH ADDED PER 0.1 GM.	SP. C. × 10⁴	VOLTS	P_H
244	0.0	27.82	0.3908	2.419
246	2.0	21.05	0.4251	3.000
248	2.5	20.21	0.4358	3.180
247	2.8	19.49	0.4485	3.395
250	3.5	21.84	0.5184	4.578
251	4.0	22.43	0.7098	7.816
252	5.0	24.93	0.8531	10.24
249	6.0	28.38	0.8970	10.98
253	7.0	32.96	0.9169	11.32
245	8.5	41.78	0.9288	11.52

TABLE XV

SPECIFIC CONDUCTIVITY INCREASE UPON ALKALINIZATION

ARSPHENAMINE	DIHYDROCHLORIDE	DISODIUM SALT	INCREASE
A	28.46 × 10⁴	41.65 × 10⁴	13.19 × 10⁴
B	28.43	38.82	10.39
C	27.82	41.78	13.96
D	29.97	42.41	12.30
E	30.82	42.44	11.62
F	27.01	41.03	14.02
G	23.20	41.24	18.04
H	27.19	39.93	12.74
I	26.88	41.96	15.08

Four types of curves were plotted from the data obtained in each series. These were: Type I, specific conductivity versus c.c. 0.1 N NaOH added per 0.1 gm. sample; type II, specific conductivity verses P_H values of the solutions; type III, specific conductivity versus NaOH required for alkalinization and

Plate I-K

Arsphenamines - A,C,F,G,H,I.

Commercial Drugs.
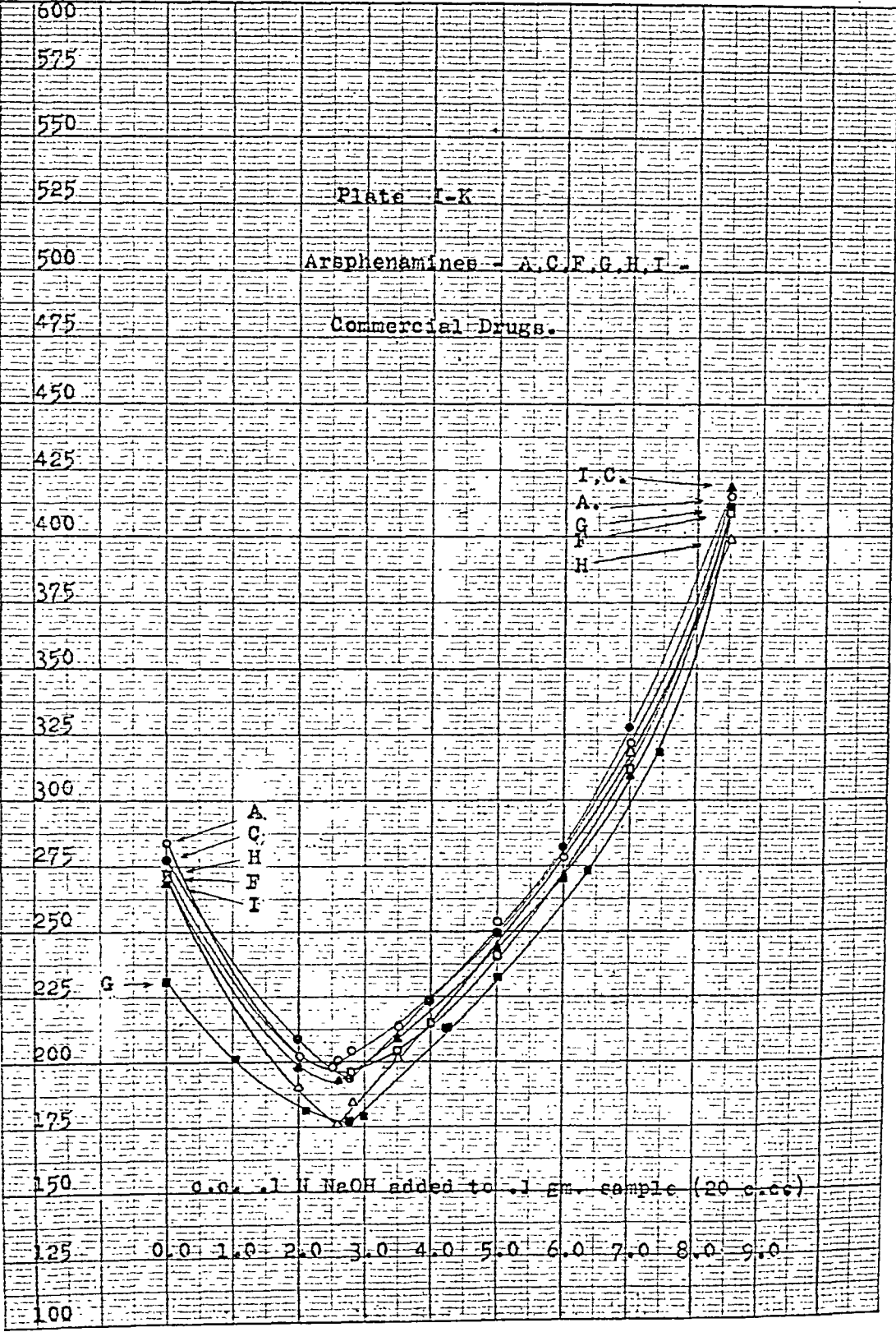
HCl added to convert the formed disodium salt back into the dihydrochloride; type IV, P_H values of solutions versus alkali and acid added as in III. The curves of types III and IV will be discussed in a later paper.

Table V gives the data of a typical series of experiments.

Plate I-C illustrates the curves of type I in showing that upon the addition of NaOH to the solution of arsphenamine dihydrochloride the specific conductivity drops to a minimum after about 2.8 c.c. 0.1 N NaOH have been added per 0.1 gm. sample. A further addition of alkali caused an increase in the specific conductivity of the solution. This was characteristic of all of the arsphenamines examined. Plate I-K indicates the phenomena as presented by the six different commercial arsphenamines used in this investigation.

The increase in specific conductivity of the respective arsphenamines upon alkalinization is shown by Table XV. It is to be noted that a marked difference occurred only in the case of arsphenamines B and G. Arsphenamine B was studied for comparative purposes because it showed visible signs of some sort of decomposition. The drug instead of being bright yellow had a noticeable brownish cast and its solution had a brownish tinge. Table XVI shows again where this material differed slightly from the other materials in that the P_H of its alkalinized solution was a bit lower. Arsphenamine G showed no signs of decomposition. It was characterized by exceptional solubility for arsphenamine. Its solution was clear and citron yellow. Since arsphenamines D and E represent the regular and the washed laboratory preparations it was thought possible that a difference might show up at this point. Such, however, was not the case.

Plate II-C shows the variation of the P_H of the solution with the specific conductivity during alkalinization. This type of curve is important because the P_H at the minimum indicated represents the isoelectric point of arsphenamine. Plate II-K gives the analogous curves for the six commercial arsphenamines studied. These appear to fall in two classes: Arsphenamines C, D and H which show a sharp break at the isoelectric point, the minimum, and arsphenamines A, E, F, and G wherein the slope of ascent is more gradual.

Table XVI shows the change of the P_H of the respective solutions during the alkalinization.

TABLE XVI

COMPARISON OF PH CHANGES
(Alkalinization)

SAMPLE	C.C. 0.1 N NaOH ADDED										C.C. 0.1 N NaOH AT MINIMUM SP. C.	I. P.
	0	1	2	3	4	5	6	7	8	8.5		
A	2.41	2.62	3.01	3.84	8.55	10.16	10.74	11.22	11.43	11.55	2.5	3.25
B	2.36	2.76	2.96	3.75	6.90	9.62	10.45	10.96	11.25	11.40	2.8	3.42
C	2.42	2.68	3.00	3.70	7.80	10.24	10.98	11.32½	11.50	11.52	2.8	3.39
D	2.40	2.63	2.81	3.58	6.40	10.14	10.68	11.10	11.40	11.56	2.8	3.41
E	2.39	2.66	2.85	3.20	6.24	10.05	10.76	11.24	11.45	11.52	2.8	3.40
F	2.43	2.62	3.06	3.65	6.67	10.14	10.70	11.00	11.36	11.51	2.8	3.39
G	2.48	2.75	3.15	4.06	9.32	10.09	10.40	10.75	11.20	11.42	2.8	3.40
H	2.35	2.58	2.96	3.85	8.46	10.32	10.88	11.24	11.45	11.54	2.6	3.40
I	2.41	2.52	2.95	3.50	6.97	10.24	10.81	11.18	11.42	11.56	2.6	3 34
J	2.41				(8.53—A, C, G, H)					11.40		
	2.47				(6.63—B, D, E, F, I)					11.27		

Plate II - C.

Arphenamine - C -

Specific Conductivity X 10^5

Region of precipitation.

pH of Solutions.

Plate II-K,

Arsphenamines - A,C,F,G,H,I -

Commercial Drugs.

Specific
Conductivity
x 10⁷

pH of Solution.
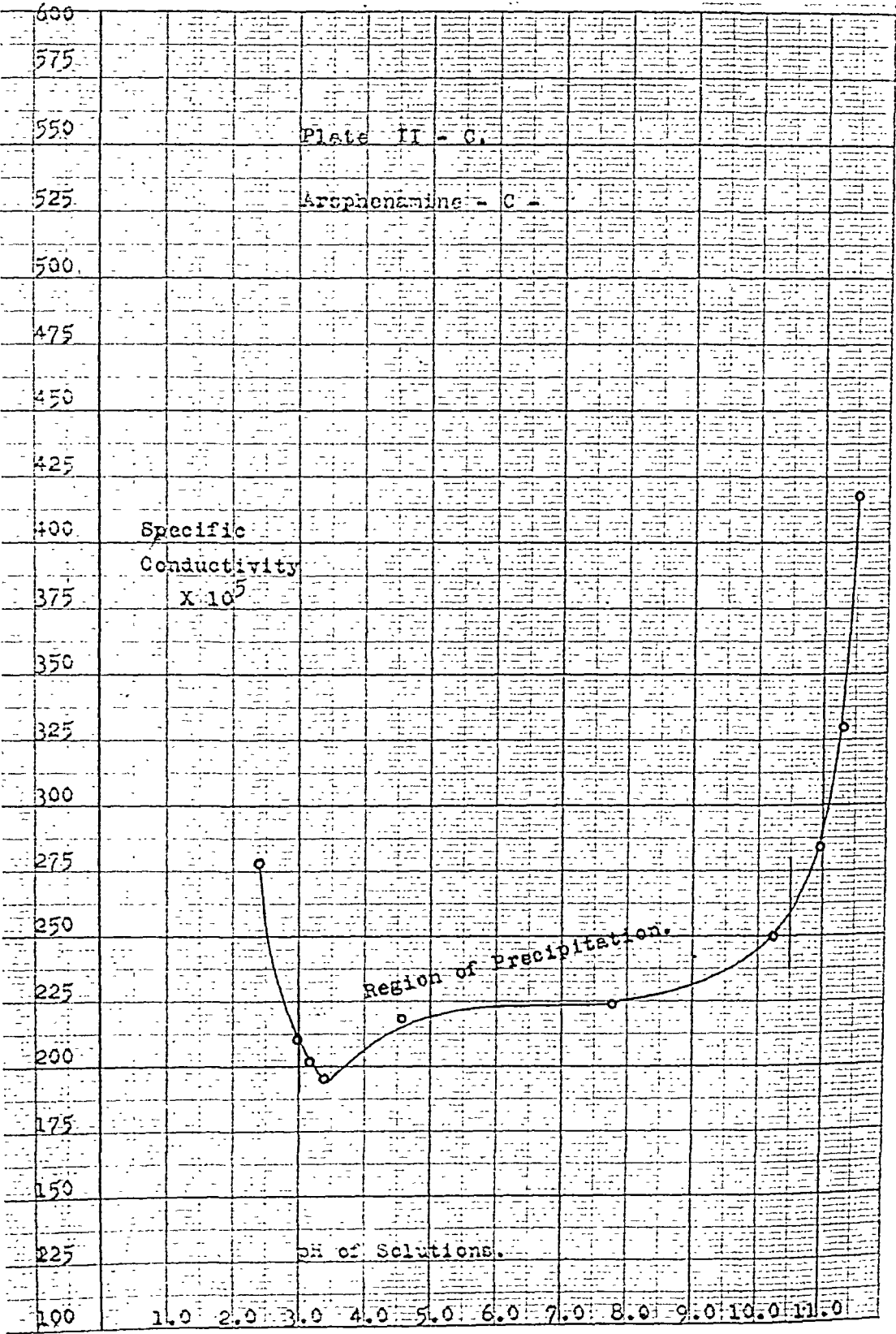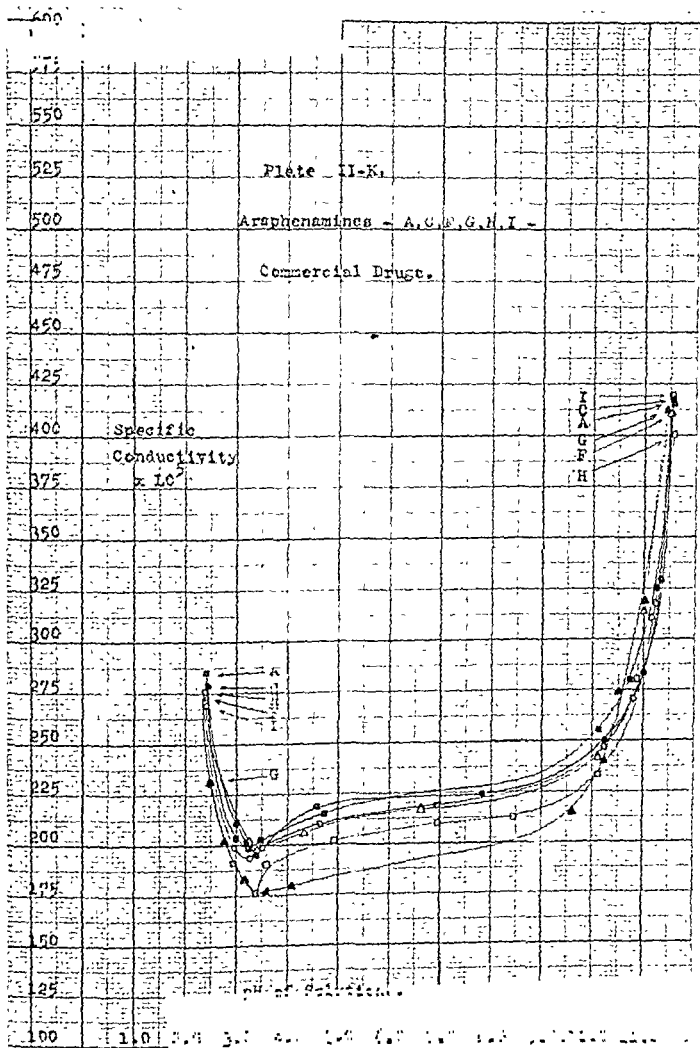
The mean value for the isoelectric point calculated from the eight normal samples studied is 3.39. The fact that this point is found so far over on the acid side indicates that the acid ionization of arsphenamine is much stronger than the basic ionization. It must be noted that the P_H values of the dihydrochloride solutions as well as of the alkalinized solutions are remarkably in agreement for the different arsphenamines. This would seem to indicate that arsphenamine, when correctly prepared, contains the same amount of acid, regardless of the method of manufacture. Indeed, when a solution of the dihydrochloride was made up and to it added 1.45 c.c. 0.1 N HCl per 0.1 gm. sample (2 molecular equivalents from the point of minimum conductivity) the P_H of the solution dropped to 2.047. This would mean that the presence of excess acid was not covered by any buffer action of the drug. An interesting thing in these curves is the slope after the minimum has been passed. It is to be observed that this rapid change in the P_H value of the solution occurred through the region of precipitation which expends approximately from a P_H of 3.0 to about 10.5 (Plate II-C). The variability of this region is further shown by Table XVI, where it can be seen that the P_H values of the comparative solutions of the different arsphenamines are very uniform with the exception of the solutions which contained 4.0 c.c. 0.1 N NaOH per 0.1 gm. These solutions are about in the middle of the precipitation region. The materials appear to fall in two groups: Arsphenamines A, C, G, and H having a mean P_H value of 8.53 while the mean value of B, D, E, F and I is 6.63. It is at this point that the most marked difference appears.

Table XVIII gives the alkalinization data for arsphenamine in terms of the P_H values. As these values are the mean of those of six different commercial drugs and two laboratory preparations they may well be taken as representative of arpshenamine.

TABLE XVIII

ALKALINIZATION TABLE FOR ARSPHENAMINE

C.C. 0.1 N NaOH ADDED PER 0.1 GM. ARSPHENAMINE	P_H VALUE OF SOLUTION
0.0	2.410
1.0	2.630
2.0	2.980
3.0	3.740
4.0	7.580
5.0	10.170
6.0	10.740
7.0	11.170
8.5	11.530

Plate VII which was made up from this data may then be taken as the alkalinization curve for arsphenamine. This shows how the P_H of the solution changes during alkalinization upon preparing a solution of the drug for intravenous injection according to the specifications of the U. S. Public Health Service.

From this information it was possible to calculate the P_H values of the solutions of the various salts of arsphenamine. The arsenic content of the preparations examined ranged from 30.80 to 32.3 per cent. Taking a mean

Plate VII

value of 31.34 per cent As. (theory = 34.17 per cent), a 1.0 gm. sample made up to 200 c.c. corresponds to a 0.014 molal solution. On this basis the P_H values of the salts of arsphenamine are as follows:

Dihydrochloride	=	2.41
Monohydrochloride	=	3.00
Arsphenamine base	=	7.60
Monosodium salt	=	10.88
Disodium salt	=	11.43

The approximate position of these points has been indicated on Plate I-C. These values are in good agreement with those of Elvove and Clark (1924). In addition they confirmed the accuracy of the hydrogen electrode measurements by the rate of hydrolysis of methyl acetate in the case of the dihydrochloride and by the rate of hydrolysis of nitrosotriacetonamine in the case of the alkalinized solution.

A general discussion will be reserved for a later paper wherein this investigation has been carried over to a study of alkalinized arsphenamine solutions.

SUMMARY

1. The hydrogen electrode measurements have been made on arsphenamine solutions and are found to give reliable and reproducible data.

2. A radiotron vacuum tube has been used as a generator of alternating current for conductivity work.

3. Eight different brands of commercial arsphenamine have been studied with the purpose of detecting differences in their physical and colloidal properties.

4. Variations of P_H were found in solutions after about two equivalents of alkali had been added. The solutions of the dihydrochloride and the alkalinized solutions showed, in general, but little variation in their P_H values.

5. A practical alkalinization curve for arsphenamine has been established in terms of P_H values.

6. A uniform, minimum conductivity measurement indicates the presence of an isoelectric point of arsphenamine at the P_H about 3.4. Reproducible conductivity measurements were made on solutions throughout the entire process of alkalinization.

REFERENCES

[1]Bauer, H., 1919: Kolloidchemische in der Salvarsanreihe, Arbeiten aus dem Inst. f. Exp. Therapie und dem Georg Speyerhause zu Frankfort a/M.No. 8, 45, Verlag Gustav Fischer, Jena.

[2]Clark, Wm. M., 1920: The Determination of Hydrogen Ions. Williams and Wilkins, Baltimore.

[3]DeForest, Lee, 1920: The Audion, its Action and Some Recent Applications, J. Franklin Inst., exc. 6.

[4]Elvove, E. and Clark, Wm. M., 1924: A Study of the Acid-Basic Equilibria of Arsphenamine Solutions, U. S. Hygienic Lab. Bul. 135.

[5]Freundlich, Stern, and Zocher, 1923: Colloidal Observations on Salvarsan and Neosalvarsan. Biochem. Ztschr., cxxxviii, 307-17.

[6]Hardy, W. B., 1900: Proc. Roy. Soc., Lond., lxvi, 110.

[7]Kraus, C. A. and Parker, H. C., 1922: The Calibration of Cells for Conductance Measurements. Jour. Am. Chem. Soc., xliv, 2422.

[8]Loeb, J., 1923: Proteins and the Theory of Colloidal Behavior, McGraw Hill, New York.

[9]Levene, P. A. and Simms, H. S., 1923: Calculation of Isoelectric Points. Jour. Biol. Chem., lv, 801.

[10]Michaelis, L., 1914: Die Wasserstoffionkonzentration, Berlin.

[11]Raiziss, Geo. W. and Gavron, J. L., 1922: Study of the Colloidal Properties of Arsphenamine and Allied Products. Jour. Pharmacol. and Exper. Therap., xx, 163.

[12]Sherndal, A. E., 1922: Arsphenamine, Some Factors which Influence Its Colloidal Properties. Jour. Lab. Clin. Med., vii. 723.

[13]U. S. Public Health Report, 1922: Preparation and Administration of Arsphenamine and Neoarsphenamine, No. 774.

[14]Voegtlin, C., Johnson, J. M., and Dyer, H., 1924: Viscosity and Toxicity of Arsphenamine Solutions. U. S. Public Health Rpts. No. 898.

FURTHER OBSERVATIONS ON A CHEMICAL FACTOR IN THE RESISTANCE TO TUBERCULOSIS*

By H. J. Corper, M.D., Ph.D., and Max B. Lurie, M.D., Denver, Colorado

IT is at present generally acknowledged that tubercle bacilli gaining entrance into the animal economy do not necessarily produce tuberculosis there, but the mechanism whereby the disease-producing propensity of this organism is prevented from manifesting itself is rather obscure. There are, however, factors, primarily of cellular nature and necessarily influenced by the virulence of the infecting bacilli which, though insusceptible of crucial experimental test, must be acknowledged as of importance as a determinative in turning infection into disease and may account for the difference in species susceptibility to tuberculosis.

The preponderance of pulmonary tuberculosis among the human races has led to many suggestions in explanation for its prevalence, viz.: a diminished resistance of the lung over other organs (a veil for our ignorance); the lung acting as a bacterial filter (Borrel, Aufrecht); the greater incidence of aerogenic infection (Cornet, Flügge); the absence or comparative weakness of pulmonary lipases; the peculiar relation of the pulmonary circulation (White); and yet none have been susceptible to crucial experimental tests nor has this been successful when tried. That the lung is especially susceptible to tuberculosis spontaneously in man and animal is an accepted universal fact, and experimentally one of the most striking evidences of pulmonary susceptibility is presented in the differentiation test for human and bovine tubercle bacilli by intravenous injection into rabbits as disclosed by the epoch-making discovery of Theobald Smith in 1898,[1] although the organ deposition following such injections was not seriously considered in these studies. Smith even states that "the lungs, as the first and chief recipients of the injected bacilli, were in a state of expansion (apparently due to tuberculosis), which in some cases was extreme," thus showing his complete failure to realize at that time the localization of the bacilli in the body. Following the intravenous injection of human tubercle bacilli the disease is limited almost exclusively to the lungs, while bovine bacilli although producing a general disease also prefer a pulmonary localization in these animals (Oehlecker, Askanazy, Donati).

In 1921, Corper, Gauss and Rensch[2, 3] pointed out that humoral antibodies have failed to explain resistance to tuberculosis; the lymphocyte as the important factor is rapidly being discarded; allergic phenomena require further elucidation, and the phagocytic action of the wandering endothelial cell is recognized as important; but both humoral and cellular mechanisms of resistance leave much to be explained. They believed the empiric reasoning that the deficiency of carbon dioxide in the body favors tuberculosis, while an accumulation retards it, seems to be highly significant. In experiments conducted at that time they found that three per cent carbon dioxide inhibits the growth of tubercle bacilli in the test tube and fifteen per cent is tuberculocidal. Cultures of tubercle bacilli buried in the tissues of animals and permitted to acquire the carbon dioxide concentration of the body are definitely inhibited in their growth, while other cultures similarly buried, except that ingress of atmospheric air is permitted, show no inhibition. It appeared from these experiments that carbon dioxide may play a significant rôle in the resistance to tuberculosis.

In 1924, Corper and Goldberg[4] reported on experiments in rabbits in which they found that the carbon dioxide content of the blood plasma obtained from a congested auricle, produced by applying mild constriction of rabbit's ears, was about 10 to 20 per cent more in terms of bicarbonate than that from normal auricles; and that tubercles resulting from the intracutaneous inoculation of graded amounts of virulent human and bovine tubercle bacilli, tended to ulcerate and discharge their contents earlier in the congested than in the noncongested auricle of the same rabbit. The development of local tubercles was retarded by prolonged constriction of the base of the auricle, with its resultant continuous mild congestion and edema. This effect was not discernible when heat-killed bacilli were used. Prolonged mild constriction of the auricle had no appreciable influence on the development of multiple metastatic lesions resulting from infection with virulent bovine bacilli nor on the development of tuberculosis in the regional lymph nodes of the ear after infection with virulent human or bovine bacilli. About this same time Levinson and Petersen,[5] reporting on the effect of passive hyperemia of the liver on tubercle formation, found that in an experimentally produced passive hyperemia of the liver, no striking differences were noted grossly between the treated and control animals as to the quantitative distribution or size of tubercles in the liver; but histologic examination of the livers showed a greater deposit of fibrous tissue around and within the tubercles in animals that received oil before and after being injected with tubercle bacilli. The lungs of the control animals contained more tubercles than the lungs of the animals receiving oil after the tubercle bacilli injection. The lungs of the rabbits receiving oil, prior to tubercle bacilli injection, were relatively free from tubercles. Admittedly these experiments are complicated by the use of the oil and the authors comment on the findings noted but offer no explanation for them.

In considering the action of particulate substances such as oil and carbon given intravenously, it seems pertinent that the distribution of these materials in the animal economy be considered. This has been fairly well studied

with various types of rapidly growing bacteria, carbon and relatively insoluble chemical substances but it seems to have been overlooked or ignored in considering bacterial infections. However, in 1908 Oettinger,[6] in studying the disposition of the lung to tuberculosis in furtherance of Flügge's views, pointed out that after entrance of tubercle bacilli into the blood stream primarily the lungs alone become diseased. This he attributed not to a greater disposition of the lung to retain the bacilli but to an increased disposition of the lung tissues to react with disease to a smaller number of bacilli. Bacteria, circulating in the blood like other corpuscular elements, are deposited in all the organs of the body but in far less amount in the lungs than in the spleen and liver. If bacilli after ingestion are found in the lungs while the liver and spleen remain free from them it is certain that they did not enter the lungs via the blood stream. Oettinger points out the erroneous conceptions held by Borrel in 1893 and Aufrecht in 1905 that the lungs acted as a selective filter for tubercle bacilli introduced into the blood which supposedly accounted for the development of the tuberculosis there.

Oettinger used rabbits to which were given intravenous injections of bacillary suspensions of B. prodigiosus, Staphylococcus albus, hog erysipelas, mouse typhoid and typhoid bacilli. Unfortunately his studies were only over a maximum of four hour intervals. The relation of the lung, liver and spleen in bacterial content was found to be extremely variable ranging from 1:1200:260, with prodigiosus, to 1:15:12 with mouse typhoid at one hour, and from 1:3:80 with prodigiosus, to 1:100·166 with typhoid at four hours interval. An attempt to study the organ distribution of tubercle bacilli after intravenous injection into the ear vein of the rabbit and using the dilution extinction method as an index to the number of bacilli with the guinea pig as test animal, yielded unsatisfactory results, since in none of the dilutions used was infection absent in the guinea pigs and the conclusions drawn were that the lung, liver and spleen were all heavily seeded with tubercle bacilli after intravenous administration. With timothy hay bacilli, however, and using plating methods the spleen and liver were found to contain twenty times as many bacilli per gram as the lung. To overcome the criticism that small numbers of bacilli may lodge mainly in the lungs, this was tried using 1000 and 10,000 B. prodigiosus but the relations still bore out the fact that the liver and spleen gave colonies while the lung usually did not.

Regarding the quantitative distribution of particulate material after intravenous administration, the recent excellent work of the Harvard investigators, Drinker and Shaw,[7] Lund, Shaw and Drinker[8] and Drinker, Shaw and Drinker,[9] furnish about the best available information and especially so, because the study included a number of different animals, viz., the dog, rabbit, guinea pig, cat, rat, chicken and turtle. Manganese dioxide and manganese metasilicate were chosen because of their ease of exact chemical determination and the natural absence from the tissues. In brief, these observers found that particles no larger than one micron disappeared completely from the blood of the cat in one hour and at that time the lungs contained 47 per cent, liver 38.3 per cent and spleen 4.3 per cent. The cat is, however, an exception, and in dogs, rabbits, guinea pigs, rats, chickens and turtles the liver receives

difficulties encountered no one seems to have reported on this phase in tuberculosis, especially from an approximate quantitative standpoint and by using several different animal species. This was the primary purpose of the study to be briefly reported here. The animals chosen were the rabbit, guinea pig and dog. The cat was not used because all available evidence indicated that the lungs in this animal were seeded more heavily with particulate matter given intravenously than the liver and spleen. On account of the difficulty encountered in determining with any degree of accuracy the numbers of tubercle bacilli in an organ and the apparently similar organ deposition of many forms of particulate matter in the same animal, carbon was chosen as a gauge for the deposition, primary and subsequent, of the tubercle bacilli injected intravenously.

THE IMMEDIATE AND FINAL ORGAN DEPOSITION OF CARBON FOLLOWING INTRAVENOUS INJECTION

For the purpose of determining the immediate and final organ distribution of carbon, guinea pigs, rabbits and dogs were given intravenous injections of India ink, in which the particles were approximately about one micron in size and by volume would give a sediment, after centrifugation at 3000 r.p.m., of about 0.2 c.c. carbon in 1.0 c.c. of original ink which was equivalent to a sediment of tubercle bacilli obtained from a suspension of 100 mg. Besides noting the gross and microscopic distribution of the carbon in the three main organs, lung, liver and spleen, which will be included in this report, the carbon content of the organs was determined colorimetrically after completely digesting the tissues with hot 40 per cent potassium hydroxide and sedimenting the carbon after two washings with an excess of 95 per cent alcohol, and further washing the sediment with two changes of ether and finally suspending the residue in 0.9 per cent sodium chloride solution, which suspension was compared in a colorimeter with a known suspension of India ink in saline. This method was accurate to 0.001 c.c. of India ink (0.000,2 c.c. of solid carbon suspension) per gram of tissue. Beyond this point, colored substances derived from the tissues themselves interfered with the readings.

By means of this method, in a series of thirty-four rabbits given intravenous injections of 3 c.c. of 1:3 dilution of India ink per pound of body weight, and analyzing the spleen, liver and lung for carbon content after intervals of thirty minutes, three hours, one and four days, one and two weeks and one month it was found that in all the above-mentioned time intervals after intravenous injection of the India ink, except the thirty minute interval, the lungs invariably contained less carbon per gram of tissue than the liver and spleen of the same animal. In the thirty minute interval, the lungs of the rabbits contained more carbon than the liver though less than the spleen per gram tissue. These relations are shown in Table I, which are the average figures obtained from the entire series of animals.

With the same dose of India ink per pound animal weight given intravenously, there was found a wide variation between individual rabbits but

TABLE I

THE RELATIVE AVERAGE DISTRIBUTION OF CARBON IN THE LUNG, LIVER, AND SPLEEN OF RABBITS
AT DIFFERENT INTERVALS AFTER THE INTRAVENOUS INJECTION OF INDIA INK*

ORGAN	AMOUNT OF CARBON (IN CUBIC CENTIMETERS OF EQUIVALENT OF ORIGINAL INDIA INK) CONTAINED IN 1 GRAM OF ORGAN ANALYZED AFTER:—						
	30 minutes	3 hours	1 day	4 days	1 week	2 weeks	1 month
Lung	0.04	0.02	0.02	0.009	0.02	0.009	0.004
Liver	0.01	0.03	0.03	0.03	0.03	0.03	0.009
Spleen	0.07	0.07	0.14	0.07	0.21	0.11	0.02

*There was noted no appreciable difference in the distribution of the carbon in these organs whether a fine suspension of carbon (particles of about 1 micron size) or slightly coarser (particles of about 3 micron size) particles were used for intravenous injection.

the relation between the carbon found in the lung, liver, and spleen was found to be almost invariable in the same animal.

Similar analyses of the organs of guinea pigs given India ink (about 4 c.c. of 1:6 dilution per pound) and analyzed after thirty minutes, three hours, one and two days, two weeks, and eight months, revealed that at the intervals examined the lungs always contained less carbon per gram weight than the liver or spleen. The ratio between the lung, liver and spleen was about 1:10:20, although this varied for the individual animal. In no case examined,

TABLE II

THE RELATIVE AVERAGE DISTRIBUTION OF CARBON IN THE LUNG, LIVER, AND SPLEEN OF GUINEA
PIGS AT DIFFERENT INTERVALS AFTER THE INTRAVENOUS INJECTION OF INDIA INK

ORGAN	AMOUNT OF CARBON (IN CUBIC CENTIMETERS OF EQUIVALENT OF ORIGINAL INDIA INK) CONTAINED IN 1 GRAM OF ORGAN ANALYZED AFTER:—					
	30 minutes	3 hours	1 day	2 days	2 weeks	8 months
Lung	0.001	trace	trace	0.006	0.007	0.002
Liver	0.02	0.01	0.01	0.01	0.01	0.005
Spleen	0.01	0.03	0.03	0.03	0.02	0.01

however, did the liver or spleen contain an equal or less amount of carbon per gram weight than the lungs. Comparatively speaking the lungs of the guinea pig contained less carbon per gram of tissue with relation to the liver and spleen than that noted for the rabbit. The averages of analyses of the organs of seventeen guinea pigs are recorded in Table II.

TABLE III

THE RELATIVE AVERAGE DISTRIBUTION OF CARBON IN THE LUNG, LIVER AND SPLEEN OF DOGS
AT DIFFERENT INTERVALS AFTER THE INTRAVENOUS INJECTION OF INDIA INK

ORGAN	AMOUNT OF CARBON (IN CUBIC CENTIMETERS OF EQUIVALENT OF ORIGINAL INDIA INK) CONTAINED IN 1 GRAM OF ORGAN ANALYZED AFTER:—					
	30 minutes	3 hours	1 day	4 days	1 week	1 month
Lung	0.03	0.01	0.03	0.03	0.05	0.01
Liver	0.05	0.02	0.03	0.04	0.05	0.04
Spleen	0.03	0.04	0.05	0.03	0.05	0.08

Analyses of the organs, lung, liver and spleen of twelve dogs given India ink (2 c.c. of 1:2 dilution in saline per pound of body weight) intravenously after thirty minutes, three hours, one and four days, one week, and one month, revealed that the lungs per gram weight tend to retain less of the carbon

than the liver and spleen, although this did not occur as consistently as in the guinea pig or rabbit. There was also lacking the striking differences between these three organs with relation to the amount of carbon retained following the intravenous injection of India ink. The liver and spleen at times assumed a reverse relationship in that the liver would retain more carbon than the spleen. However, in all the animals, rabbits, guinea pigs and dogs, there was a gradual removal of the carbon from all three organs as time went on, although this occurred very slowly. The average of the analyses at the different intervals for the dogs is recorded in Table III.

Considering the fact that the guinea pigs received intravenously about two-thirds c.c. of full strength India ink per pound, the dog and rabbit 1 c.c. per pound, it is possible to compare in a general way the relative pulmonary

Fig. 1.—Distribution of tuberculosis in the guinea pig four weeks after the intravenous injection of 0.1 mg. virulent bovine tubercle bacilli. Note the absence of liver and pronounced pulmonary involvement.

localization in these animals; the dog's lung received most, the rabbit's lung an intermediate amount and the guinea pig's lung the least on the basis of 1 gram tissue weight. However, in all these figures the amount of carbon in the actual lung volume is exaggerated since 1 gram lung tissue considerably exceeds the v⸱   f 1 gram liver or spleen tissue.

THE TUBERCUL⸱   VEMENT OF VARIOUS ORGANS FOLLOWING THE INTRAVENOUS
INJECTION OF TUBERCLE BACILLI

For the p⸱   studying th   ⸱rculous involvement in rabbits following the int⸱   ⸱jection of   ⸱sions of tubercle bacilli, thirty rabbits were give⸱   0.01 mg⸱   01 mg. and 0.000,000,1 mg. of a

uniform suspension of virulent bovine tubercle bacilli. The bovine tubercle bacillus was used in rabbits because of the comparative greater susceptibility of these animals to the bovine bacillus over the human bacillus, and the acknowledged general selective pulmonary election of the human bacillus following intravenous injection in these animals. The animals were examined at various intervals from two weeks to three months after intravenous injection.* In the 1.0 and 0.01 mg dose of bacilli twelve of fourteen rabbits revealed
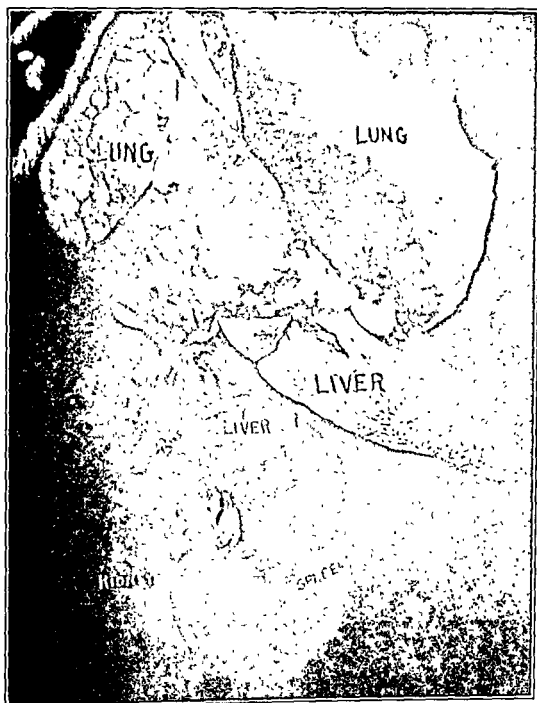
Fig. 2.—Distribution of tuberculosis in the rabbit eleven weeks after the intravenous injection of 0.000,01 mg. of virulent bovine tubercle bacilli per pound body weight   Note marked pulmonary involvement, slight splenic involvement and absence of hepatic involvement.

a massive involvement of the lungs and of these only one showed a slight macroscopic tuberculosis of the liver, and the spleen was involved slightly or moderately in seven of the twelve rabbits, while the kidneys were slightly to moderately involved in eight. In the remaining two rabbits there was a

*For lack of space the elaborate tabulated data on the tuberculous involvement of the rabbits, dogs and guinea pigs following the intravenous injection of tubercle bacilli will be recorded more fully in a subsequent article elsewhere.

miliary pulmonary tuberculosis with no macroscopic involvement in any of the other organs examined. Of the sixteen rabbits that received 0.000,01 and 0.000,000,1 mg. of bacilli, the lung was massively involved in three of the animals, while the liver revealed a slight involvement in only one of these, the spleen of two was moderately involved and one had a slight involvement. The lungs of four rabbits contained numerous large individual tubercles and in only one of these was the liver moderately involved while the spleen was only slightly involved in all four. The lungs of two rabbits were moderately involved with no hepatic or splenic involvement macroscopically. The lungs

Fig. 3.—Distribution of tuberculosis in the dog thirteen weeks after the intravenous injection of 0.001 mg. of virulent bovine tubercle bacilli per pound body weight. Note presence of pulmonary miliary tubercles and absence of involvement of the spleen and liver.

of seven rabbits were slightly involved with splenic involvement in one case only.

The experiments in rabbits indicate the remarkable susceptibility of the lung to tuberculosis as contrasted with the remarkable resistance of the liver in this animal, the spleen being intermediate in susceptibility. It was also noted that the kidney was involved in the same number of animals as the spleen. The size of the tubercles in these two organs tended to be the same— the difference between the number of tubercles in the spleen and kidneys being accounted for by the relative seeding. The spleen in the rabbit does not enlarge greatly when involved with tuberculosis, while in the guinea pig it does.

In order to study the tuberculous organ involvement in guinea pigs following the intravenous injection of suspensions of tubercle bacilli forty-four guinea pigs were given 0.1, 0.001, 0.000,01 and 0.000,000,01 mg. of a suspension of virulent bovine tubercle bacilli and groups of these animals were killed two, four and six weeks after injection. In addition, twelve guinea pigs were given intravenous injections of a suspension of virulent human tubercle bacilli in doses of 0.1, 0.001, 0.000,01 and 0.000,000,01 mg. and were examined two, four and six weeks after injection. From a critical study of the data
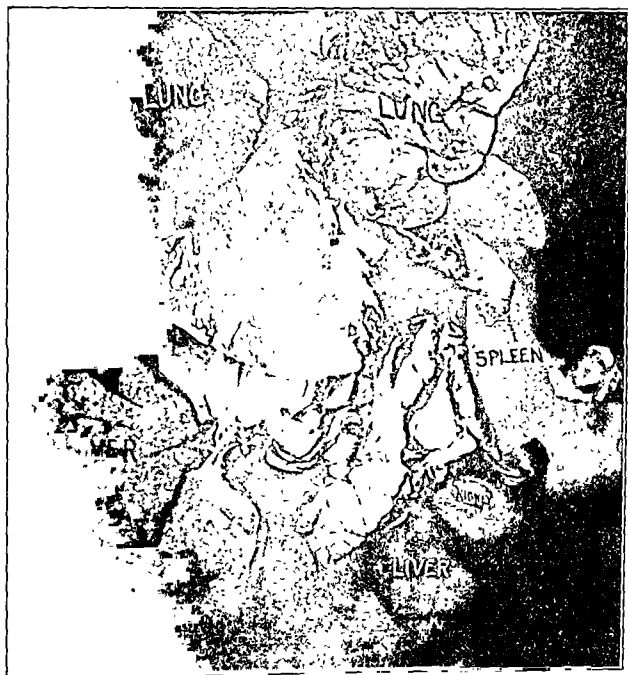
Fig. 4.—Distribution of tuberculosis in the dog seven weeks after the intravenous injection of 0.001 mg. virulent human tubercle bacilli per pound body weight. Note the larger size of the miliary tubercles in the lung, in distinction to the greater number of those in the liver; the number apparently indicating the relative seeding of the bacilli in these two organs when both organs develop the disease

obtained from these animals having received human or bovine bacilli, it was noted that during the first two weeks macroscopic tubercles appeared in the lungs of seventeen of twenty guinea pigs without macroscopic evidence of their presence in the liver; in the remaining three, slight involvement occurred but the tubercles were much smaller than those seen in the lungs of the same animals. After four weeks the guinea pigs fall into three classes,

those having received 0.1 and 0.001 mg. of bacilli uniformly had more macroscopic tuberculosis in the lungs than in the liver while those having received 0.000,01 mg. had more macroscopic tuberculosis in the lungs in three of five animals, while one had the same amount and one appeared to have more in the liver, and those having received 0.000,000,01 mg. had approximately the same number of tubercles in both lung and liver but in all the guinea pigs examined after four weeks the individual pulmonary tubercles were larger in seventeen of twenty animals than the corresponding tubercles in the liver; in two the size was the same and in one the tubercles in the liver were larger, which exceptions occurred only in the smaller doses. The tendency seems to be borne out that the more the pulmonary involvement the less the liver involvement. All the guinea pigs receiving 0.1 mg. of bovine bacilli died before the six weeks interval but those having received the human bacillus lived for the entire interval of time. Of the bovine virulent set at six weeks, three of four receiving 0.001 mg. had more hepatic than pulmonary involvement, three of four receiving 0.000,01 mg. and one of four receiving 0.000,000,01 mg. With the human bacillus the lung was selectively involved in all doses except in the smallest dose (0.000,000,01 mg.) in which case the number of tubercles were approximately equal but the pulmonary tubercles were distinctly larger. After six weeks, there was a tendency for the development of more tuberculosis in the liver than in the lung when virulent bovine bacilli were injected. With the human bacillus there was still more tuberculosis in the lungs even after six weeks.

The experiments on dogs included a series of thirty dogs, twenty-two given intravenous injections of 0.5 mg., 0.1 mg., 0.001 mg., 0.000,01 mg. of virulent bovine tubercle bacilli per pound weight and eight dogs were given the same doses of virulent human tubercle bacilli. The animals were examined at intervals of twenty-two days to 146 days after the intravenous injection of suspensions of the bacilli.

In every case of the thirty dogs the individual macroscopic tubercles in the lungs were larger than the macroscopic tubercles in the liver of the same dog. In twenty of the thirty dogs the total number of tubercles for a given area of lung were more numerous than a corresponding area in the liver of the same dog. In six of the thirty dogs, the liver contained more numerous tubercles than the lung of the same dog. The six dogs in which the tubercles were more numerous in the liver than in the lungs would seem to verify the distribution law established with carbon for tubercle bacilli. In fifteen of the thirty dogs, there was no macroscopic tuberculosis in the liver while there was definite macroscopic tuberculosis in the lungs.

DISCUSSION AND CONCLUSIONS

All the evidence available to date indicates that the distribution of particulate material of sufficiently small size not capable of producing capillary embolism following the intravenous injection into guinea pigs, rabbits and dogs, favors deposition in the liver and spleen of these animals as compared to the lung, both as to optical appearance and according to chemical analyses on the basis of unit weight (or volume) of organ examined. On purely *a priori*

reasoning, and in the absence of contradictory evidence, it seems that tubercle bacilli, in fine suspension injected intravenously, deposit in these organs in relatively the same proportions. If tuberculosis develops at all in the organs of these animals, it develops only in those organs which contain macroscopic carbon and an appreciable amount by chemical analyses. When the liver of these animals does not exercise a complete inhibitory action on the development of the tubercle bacilli in this organ, the relation between the number of tubercles in the lungs and liver closely correlate the deposition findings with carbon. Krause[20] has concluded from anatomic studies that the lung of the guinea pig retains less tubercle bacilli than that of the rabbit, following entrance of the bacilli into the circulation. This fact is directly borne out by the deposition of the carbon in our studies and the subsequent development of the tuberculosis in these two animals in that the relation between the liver and lung deposition in the rabbits is about 3 to 2, while in the guinea pig it is about 10 to 1, and similarly for the tuberculosis, although this is complicated by other factors.

It is also noteworthy that the microscopic study of all the organs reveals that the localization of the early tubercles in the lungs, liver, spleen and kidney is the same as the deposition of the carbon following intravenous injection. This is also borne out by the reports of the investigators who studied the immediate distribution of the rapidly growing bacteria following intravenous injection.

Therefore, it seems justified to conclude that the distribution of carbon following intravenous injection is a good approximate index of the distribution of the tubercle bacilli following intravenous injection.

If this is admitted there is a fundamental discrepancy between the organ development of tuberculosis and the bacillary distribution following intravenous injection. In the rabbit, while the bacilli are deposited in definitely smaller numbers in the lungs than in the liver and spleen, the tuberculosis, when it develops following the intravenous injection of human or bovine tubercle bacilli, develops in the lungs to a far greater extent than in the liver and spleen; in fact it is rare to have macroscopic tuberculosis develop in the liver of these animals, while the spleen occupies an intermediate position between these two organs in amount of tuberculous involvement. In the guinea pig, especially during the early periods of infection, two and four weeks, the evidence again is in favor of a greater susceptibility of the lungs of these animals over the liver. This general rule of greater pulmonary over hepatic susceptibility to tuberculosis also is borne out by the studies on the dogs. These observations are in entire accord with the conception of Oettinger of a special disposition of the lungs to tuberculosis.

Attempts to explain pulmonary disposition to tuberculosis are numerous, including the obviously wrong conception of increased deposition of bacilli in this organ following entrance into the blood stream, a virulence destroying property of this organ,[21] and the lack of pulmonary lipases. It is conceivable that cellular factors might explain the relative organ differences in these animals but the striking pulmonary susceptibility in distinction to the hepatic resistance in all of them and the intermediate uniform tuberculosis of the

kidney and spleen—especially in the rabbit and dog—dependent upon the primary deposition of the bacilli following intravenous injection, would seem to indicate that another factor is active in determining the results in all the animals studied, which is the same for all of them. That the greater motility of the lung over all the other organs does not enter in this is well established by the fact that unilateral artificial pneumothorax in the rabbit had no effect upon pulmonary miliary tuberculosis.[22] While all the organs considered vary as to their cellular structure, the lung and liver have one common variable and this is the blood supply. The lung is primarily an oxygenating and decarbonating organ for the body and as such distinguishes itself from all the other organs, while the liver, having essentially a venous circulation has the richest carbon dioxide and poorest oxygen supply of any organ in the body. In the light of the previous work on carbon dioxide and on the effect of constriction of the ear of the rabbit on tuberculosis developing in this appendage, it seems highly probable that this factor may be instrumental in determining pulmonary susceptibility to tuberculosis as opposed to hepatic resistance to the disease. In how far the oxygen content of these organs plays a rôle is not determined at present but is being studied. The fact, however, must not be lost sight of, that there is another important factor in tuberculosis accounting for a difference in species susceptibility which is also strikingly brought out by this study but which at present cannot be explained on a chemical basis.

From this point of view some of the most important facts in tuberculosis are easily understood—for instance, in the first place, the preponderance of pulmonary tuberculosis and relative rarity of other organ involvements in man and animals; and secondly, the age incidence and general conditions under which tuberculosis develops. Tuberculosis is a disease of early adult life, the time of greatest stress and strain associated with malnutrition, exhaustion and fatigue, all of which lower the alkali reserve of the body and thus its carbon dioxide retaining power. In the third place, rest and good nutrition, factors universally accepted as favoring the arrest of the disease, tend to increase the alkali reserve and hence the carbon dioxide combining power of the body. Fourth, the reduced incidence of tuberculosis among the poorer classes of society during the past hundred years can be correlated with the general improved living conditions of the people, shorter working hours and better nutrition; and fifth, the particular susceptibility to tuberculosis of individuals suffering from debilitating diseases—the diabetic is a classical example with lowered alkali reserve—and opposed to this the resistance to tuberculosis of the pregnant woman and those afflicted with certain cardiac and asthmatic conditions.

REFERENCES

[1]Smith, Theobald: A Comparative Study of Bovine Tubercle Bacilli and of Human Bacilli from Sputum, Jour. of Exper. Med., 1898, iii, 451-511.
[2]Corper, H. J., Gauss, Harry and Rensch, O. B.: Resistance to Tuberculosis—A Non Immunologic Chemical Factor Worthy of Consideration, Jour. Am. Med. Assn., 1921, lxxvi, 1216-8.
[3]Corper, H. J., Gauss, H., and Rensch, O. B.: Studies on the Influence of Carbon Dioxide on Resistance to Tuberculosis. The Effect of Carbon Dioxide on the Tubercle Bacillus, Am. Rev. Tuberc., 1921, v, 562-87.

[4]Corper, H. J., and Goldberg, Max: The Effect of Artificial Circulatory Constriction on Local Tuberculosis in the Rabbit, Amer. Rev. Tuberc., 1924, viii, 554-69.

[5]Levinson, S. A., and Petersen, William F.: The Effect of Passive Hyperemia of the Liver on Tubercle Formation, Jour. Am. Med. Assn., 1923, lxxxi, 723-7.

[6]Oettinger: Die Disposition der Lunge zur Erkrankung an Tuberkulose, Ztschr. f. Hyg. u. Infectionskrankh., 1908, ix, 557-71.

[7]Drinker, Cecil K. and Shaw, Louis, A.: Quantitative Distribution of Particulate Material (Manganese Dioxide) Administered Intravenously to the Cat, Jour. Exper. Med., 1921, xxxiii, 77-98.

[8]Lund, Charles C., Shaw, Louis A., and Drinker, Cecil K.: Quantitative Distribution of Particulate Material (Manganese Dioxide) Administered Intravenously to the Dog, Rabbit, Guinea Pig, Rat, Chicken and Turtle, Jour. Exper. Med., 1921, xxxiii, 231-8.

[9]Drinker, Cecil K., Shaw, Louis A., and Drinker, Katherine A.: The Deposition and Subsequent Course of Particulate Material (Manganese Dioxide and Manganese Meta-Silicate) Administered Intravenously to Cats and to Rabbits, Jour. Exper. Med., 1923, xxxvii, 829-50.

[10]Wislocki, George, B.: On the Fate of Carbon Particles Injected into the Circulation with Especial Reference to the Lungs. Am. Jour. Anat., 1921, xxxi, 423-45.

[11]Corper, H. J., Starry, A. C., and Lurie, Max B.: The Effect of Carbon on the Tubercle Bacillus in Vitro and on Experimental Tuberculosis in the Rabbit, Am. Rev. Tuberculosis, 1924, viii, 530-53.

[12]Nagao, K.: The Fate of Killed Non Hemolytic Streptococci Injected into the Blood and the Resulting Cellular Changes, Jour. Infect Dis., 1920, xxvii, 327-62.

[13]Nagao, K.: The Fate of India Ink Injected into the Blood. I. General Observations, Jour. Infect. Dis., 1920, xxvii, 527-38.

[14]Nagao, K.: The Fate of India Ink Injected into the Blood II. The Formation of Intracellular Granules and Their Movements, Jour. Infect. Dis., 1921, xxviii, 294-316.

[15]Bartlett, C. J., and Ozaki, Y.: The Fate of Micrococcus Aureus Introduced into the Blood Stream of Dogs, Jour. Med. Research, 1917, N.S., xxx, 465-86.

[16]Ito, Hiidzu: The Fate of Staphylococcus Progenes Aureus Introduced Intravenously into Actively Immunized Dogs; and of Killed Bacteria Introduced Intravenously into Normal Dogs, Jour. Med Research. 1917, N.S., xxxi, 189-207

[17]Hopkins, J. Gardner, and Parker, Julia T.: The Effect of Injections of Hemolytic Streptococci on Susceptible and Insusceptible Animals, Jour Exper. Med., 1918, xxvii, 1-26.

[18]Corper, H. J., Lurie, M. B., and Kretschmer, O. S.: Effect of Guinea Pig Tissues in Vitro Upon Virulent Tubercle Bacilli. Am. Jour. Hyg., 1924, iv, 77-100.

[19]Neumann, Wilhelm, and Wittgenstein, Hermann: Das Verhalten der Tuberkelbazillen in den verschiedenen Organen nach intravenoser Injektion. Wien. klin. Wchnschr., 1906, xix, 838-60, and Beitr z. Klin d. Tuberk., 1909, xiii, 146-199.

[20]Krause, Allen K.: Experimental Studies on Tuberculous Infection, Am. Rev. Tuberc., 1922, vi, 1-20.

[21]Corper, H. J.: The Virulence of the Tubercle Bacilli Isolated from the Sputum. Jour. Infect. Dis., 1918, xxiii, 493-503

[22]Corper, H. J., and Rensch, O. B.: The Effect of Prolonged Pneumothorax Upon Tuberculosis of the Lungs of Rabbits, Following the Intravenous Injection of Tubercle Bacilli, Am. Rev. Tuberc., 1920, iv, 763-8.

ON THE RELATIVE SIGNIFICANCE OF A PARTIAL COMPLEMENT-FIXATION TEST WITH THE CHOLESTERINIZED ANTIGEN*

By D. M. Sidlick, M.D., and A. Strauss, M.D., Philadelphia, Pa.

THE practical application of the complement-fixation test by Wassermann, Neisser and Bruck[1] to the diagnosis of syphilis has successfully passed through the era of enthusiasm and the era of pessimism that followed, to establish itself as a specific reaction. And while it is accepted that a Wassermann test that exhibits complete inhibition of hemolysis is diagnostic of syphilis, we cannot bring ourselves to believe that a partial inhibition of hemolysis, especially +1, does not require or deserve a second thought.

It is not our desire to maintain that a partial complement-fixation test, particularly one where an alcoholic extract of a tissue antigen has been reinforced with cholesterin, would authorize the physician to entertain the deduction of the existence of syphilis in an individual whose physical examination and history do not begin to suggest that the patient is afflicted with syphilis. Nevertheless, inasmuch as syphilis is known at times to manifest itself in the disguise of some malady, the etiology of which is obscure; that histories as related by patients, although without intent to deceive, are often misleading, then a weakly positive serologic reaction which in the course of the study of the patient's case, is confirmed by relevant facts which bear witness that the patient's ailment is syphilis, assumes significance. That the test although only 1+ in intensity and performed with cholesterin antigen may possess diagnostic value, that it is not pure presumption but is susceptible to concrete demonstration, is exceedingly well exemplified by the case histories here presented.

CASE 1.—M. J., white female, aged thirty-one years, housewife by occupation, was registered at the Gynecological Outpatient Department, May 6, 1922, she complained of hematuria.

Personal History.—Usual diseases of childhood. She began to menstruate at age of fourteen, married when seventeen years old and has had three apparently healthy children, aged thirteen, eight and three years respectively. Labor was normal. She denied luetic infection.

Present Illness.—In the summer of 1917, the patient noticed that there was some blood in the urine. She consulted a physician who irrigated the bladder and the bleeding "stopped." Thereafter the patient noticed the appearance of blood in the urine from time to time until several weeks prior to applying for treatment when the hematuria became constant.

Physical Examination.—The patient was fairly well nourished. The various examinations including the x-ray, the examinations upon the spinal fluid and the blood chemistry were negative. The cystoscopic examinations were made by Dr. H. Ginsberg who reported as follows:

June 7, 1922, Mild infection of the trigone. Superficial ulcerations near the fundus. June 13, 1922, blood in the bladder. Both ureters catheterized. Bloody urine from left kidney. Urine from the right side clear. A routine Wassermann test was reported +1 with the cholesterinized antigen. The patient was referred to the dermatologic clinic where she was given a provocative test. Following this test the Wassermann reaction was reported +3.

The husband was then interviewed, but denied having been infected with syphilis. He submitted to a physical examination and a Wassermann test on the blood. The medical examination was negative but the Wassermann test was +4. When confronted with the positive test he admitted having had a chancre four months prior to his marriage. The oldest child was also examined and was found also to have a +4 Wassermann reaction.

Course.—Following the provocative test the hematuria cleared up. The patient has received four full courses of treatment—each course consisting of 4.8 gm. of neoarsphenamino and 12 gr. of mercury salicylate. At present the Wassermann test is negative. The patient since has given birth to a child which is physically and serologically negative.

CASE 2.—E. C., colored, female, aged eighteen years, referred to skin clinic from the Gynecological Outpatient Department where she was receiving treatment for gonorrhea. A routine Wassermann reaction was +1 with the cholesterinized antigen.

Following the provocative test, the serologic reaction was reported +4. The medical examination from the viewpoint of syphilis was negative. Consent for spinal drainage was refused.

Course.—The patient was irregular in attendance. The last test on the blood which was made in 1923 was still 4+.

CASE 3.—N. B., Italian, laborer by occupation, registered at skin clinic in 1921 with the express purpose to have his blood tested for syphilis, because, as he stated "one of the roomers at the boarding house was being treated for syphilis." The serologic examination on the blood was reported 1+ with the cholesterinized antigen. The routine medical examination was negative. The examinations on the spinal fluid were reported as follows: Wassermann reaction +4, cell count 31 per c.c., definite increase of globulin. Copper sulphate solution was reduced. The neurologic examination of the patient was made after findings on spinal fluid were known. The report of the neurologist was to the effect that the patient was free from mental and physical stigmata of syphilis.

Course.—Following the first treatment the intensity of the Wassermann reaction was reported 3+. After two years' treatment the blood Wassermann is negative. The Wassermann reaction on the spinal fluid is +1, cell count 7 per c.c., the globulin is not increased.

We believe that it would not be inappropriate to review briefly the events that led to the evolution of the use of cholesterinized antigen without discussing the subject of the Wassermann test at length. At present the serologic reaction is regarded as a synonym for the complement-fixation test for syphilis and is based upon the total or partial arrest of the otherwise inevitable destruction of the erythrocytes. When first formulated by Wassermann and his coworkers they believed that the test was a specific biologic reaction, between antibody and antigen. However when Marie and Levaditi,[2] Landsteiner[3] and Weil[4] were successful in obtaining an antigen from a large variety of normal tissues and even tumors, they demolished the belief that the reaction, from the standpoint of biology, is specific in nature. Wassermann then professed to believe that the reaction constituted a serum diagnosis of an antibody-like substance, the activity of which determines the fixation of the complement. The published researches of Landsteiner, Muller and Potz,[5] Porges and Meier,[6] in which they found that they could prepare an antigen by alcoholic extract from syphilitic and normal tissues, and that

their antigens possessed antigenic properties equal to those antigens that were prepared by extraction of syphilitic liver with physiologic salt solution, gave impetus to the study of substances other than tissue extracts that would have similar or superior antigenic properties.

A multitude of substances were experimented with. Fleishman[7] suggested the use of cholesterin. However it remained for Sacks[8] to make successful application of this particular lipoid substance. He was the first to add cholesterin to alcoholic tissue extracts and the results achieved gave Sacks the impression that in many respects the new antigen was the long sought and desired factor which made possible the achievement of a standard test. The superiority of cholesterinized antigen over the previously used antigens was soon confirmed by many independent investigators.[9]

The advent of protocols, in the medical literature, enumerate almost a legion of diseases other than syphilis that gave a positive serologic reaction; the admonitions that the cholesterinized antigen is too "sensitive" and is responsible for many false positives,[10] and the discrepancies in the reports of different laboratories on the specimen of blood[11] or the divergent reports by serologists on different specimens of blood from the same patient, conveyed the hasty and immature impression to the physicians at large that the Wassermann reaction was not only worthless but that it was misleading. Yet the irresistible conclusion that stands out in bold relief, after the study of these reports, is that none of the authors,[11] except one,[12] questioned the specificity of the reaction as far as it relates to syphilis. Furthermore as the test is being perfected the diseases that were supposed in the past to have been associated with a positive complement-fixation test, one by one have been crossed off the list. The last disease to lose this distinction is leprosy.[13] Yaws remains the only other entity besides syphilis where the blood persistently exhibits inhibition of hemolysis. The belief that the cholesterinized antigen is the underlying cause of falsely positive reports has been principally based upon lack of correlation between the clinical findings and the negative history on one hand and the positive serologic findings on the other. At this point of the discussion, it would not be irrelevant to point out that the attitude of the clinician who assumes that he is infallible in the matter of the diagnosis of syphilis is not always supported by the pathologist. Cases are on record, where neither histories nor the physical examinations have alluded to the possibility of the presence of syphilis, and yet, Treponema pallidum has been demonstrated histologically to be present in the tissues.[14] The fear that the contradictory reports on the same specimen of blood by different serologists or by the same serologists on repeated specimens from one patient, and which constitutes one more indictment against the reliability of the reaction, is not founded upon fact. It is well to note that the reports in themselves are consistent.[11] The laboratory rendering a positive report on a specimen of blood invariably rendered the same report on the repeated tests carried out on the same individual. This holds true likewise when a negative result was rendered.

Only in the matter of a doubtful or 1+ reaction have the reports been conflicting. The latter inconsistencies are more apparent than real. It is common knowledge that during the phenomenon classed as latent syphilis,

in the course of the disease a patient may harbor the Treponema pallidum in the spleen, the marrow, the lymph nodes, semen, the testicles, and the material capable of transmitting the disease when used for inoculating purposes.[15] Nevertheless, the antibody-like substance that determines the fixation of the erythrocytes may be so small in amount in the circulating blood of the patient that the intensity of the reaction may vary and the report may read 1+ with cholesterinized antigen and at the same time be reported negative with the alcoholic extract. The factor most commonly responsible for the occurrence of the latter phase is the antigen employed. Hence, the clinical interpretation of the value of a positive or negative test, regardless of its intensity, is largely a matter of one's knowledge of the value and significance of the technic used.

The outline of the Wassermann test,[16] as it is done under the direction of B. L. Crawford, Clinical Laboratory, Jefferson Hospital, is appended to avoid ambiguity.

The antisheep-hemolytic system is used, and the technic consists in using inactivated serum in 0.2 c.c. amounts. A solution of complement of constant strength is used, against which the amboceptor is titrated every day, and two units of amboceptor are used in the test. Two antigens are used, cholesterinized and alcoholic extract of syphilitic liver; however, the specific antigen is not used with all the serums tested each time, but both antigens are used with a sufficient number of serums each time to have a control on the cholesterinized antigen. The first incubation of the mixture of serum, antigen and complement is done in a water-bath at 37° C. for one hour. Then 1 c.c. of a 1:40 dilution of washed sheep cells and two units of amboceptor are added separately and incubated for one hour. At the end of this time, readings are taken if the negative controls show complete hemolysis and positive controls show complete fixation.

As a matter of precaution, the tubes are put in the ice box overnight and a final reading is taken the following morning.

SUMMARY

The purpose of this paper is to emphasize the importance of a 1+ Wassermann reaction with the cholesterinized antigen, but not to maintain that this test per se is diagnostic of syphilis.

Experimental and postmortem evidence is not lacking that a patient may be afflicted with syphilis; that he may be a source for transmission of the disease and yet not manifest the disease clinically.

To have adopted the dominating view that there is no need to attach significance to a partial cholesterinized serologic reaction, the patients previously mentioned, one suffering from syphilis of a kidney, another having latent syphilis, and the third, obviously having asymptomatic neurosyphilis, would have been assured, in view of the fact that their physical examinations and histories were negative, that syphilis was not the cause of their ailments and left to the ravages of the disease.

REFERENCES

[1]Wassermann, Neisser and Bruck: Wien. klin. Wchnschr., 1906, xxxii, 745.
[2]Marie and Levaditi: Ann. de L' inst. Pasteur, 1908, p. 138.
[3]Landsteiner: International Kongress f. Hygiene u. Dermographe, 1907, Berlin, ii, 118.
[4]Weil: Wien. klin. Wchnschr., 1907, p. 527.
[5]Landsteiner, Muller and Poetz: Wien. klin. Wchnschr., 1907, xx, 1565.
[6]Porges and Meier: Berl. klin. Wchnschr., 1008, xlv, 731.
[7]Fleishman: Berl. klin. Wchnschr., 1908, p. 49.
[8]Sacks: Berl. klin. Wchnschr., 1911, xlviii, 2066.
[9]McIntosh and Fields: Ztschr. f. Chemotherap., 1912, i, 1.
[10]Walker and Swift: Jour. Exper. Med., 1913, xviii, 75.
  Kolmer, Laubaugh, Casselman and Williams: Arch. Int. Med., 1913, p. 660.
  Field: Jour. Am. Med. Assn., 1914, lxii, 1620.
  Judd: Jour. Am. Med. Assn., 1914, lxiii, 313.
  Kolmer and Schamberg: Am. Jour. Med. Sc., 1915, cxlix, 365.
  Walker: Arch. Int. Med., 1914, xiv, 563.
  Thomas and Ivy: Am. Med. Jour., lxii, 363.
[11]Walbarst: New York Med. Jour., 1913, xcvii, 378.
  Pusey: Jour. Med. Assn., 1913, lxi, 921.
  Stone: New York Med. Jour., 1914, xcix, 1242.
  Walbarst: Interstate Med. Jour., 1915, xix, 2.
  Uhle and MacKinney: Jour. Am. Med. Assn., 1915, lxv, 863.
[12]Coca: Quoted in the Bull. Johns Hopkins Hosp., 1916, xxvii, 153-155.
[13]Kolmer and Lucke: Arch. Dermat. and Syph., 1924, ix, 181.
[14]Warthin: Jour. Am. Med. Sc., 1916, clii, 508.
[15]Eberson and Engman: Am. Med. Assn., 1921, lxxvi, 160.
  Niesser, quoted by Thom, Lea and Febiger, Syphilis, 1922, p. 38.

POLLEN SENSITIZATION: A PRELIMINARY REPORT*

By J. H. Black, M.D., Dallas, Texas

THOSE who are engaged in the prophylactic treatment of hay-fever are well aware of the fact that satisfactory results are not sufficiently constant to justify one in stating that the treatment of this condition is on a stable basis. While complete freedom from symptoms is often obtained, there are large numbers of individuals whose improvement is moderate or slight and, since there is no clinical criterion by which it may be foretold into which class a given person may fall, the conscientious practitioner must be guarded in the amount of relief promised.

The difficulty in obtaining satisfactory clinical results may be due to, (1) Unsatisfactory material used in treatment, (2) Unsatisfactory method of administration of treatment, (3) Insufficient treatment, or (4) The conception of the mechanism of the condition may be fundamentally unsound. The work here presented deals only with the first and last of these possibilities.

The most generally accepted opinion of the underlying mechanism of hay-fever seems to be that it is an anaphylactic phenomenon—a response to the introduction of a pollen protein to which the individual has, in some manner, become sensitized. The arguments which have been advanced against this theory are (1) that anaphylaxis, as we know it in the experimental

animal, is not known to exist naturally, whereas hay-fever is very common, affecting possibly 1 per cent of the population[1]; (2) anaphylactic animals may be rendered refractory to subsequent introduction of the specific protein by a single injection of the protein. This has not been found possible in hay-fever. (3) The anaphylactic state may be passively transferred to normal animals while the very few reports of such transference in hay-fever are probably open to question.

STANDARDIZATION OF POLLEN EXTRACTS

In attempting to secure some information relative to the question of of anaphylaxis as the possible basis of hay-fever, it was recognized that in work extending over a period of months requiring the use of more than one pollen extract, some definite knowledge of the actual content of active substance in these extracts must be obtained and some means of comparing the value of these extracts in terms of active substance must be had. The first work done, therefore, was an investigation into the question of standardization of extracts.

The clinical criterion of activity in general use has been the skin reaction of the hay-fever patient. An inherent difficulty, generally recognized, is the wide variation in reaction of the skin of different individuals which makes the method wholly inadequate for quantitative work. It is also a possibility which has, apparently, not always been kept in mind, that an individual who has been treated with a pollen extract may give a reaction to the protein of the extract used in the test which may have no relation to the reaction due to the *active substance* of the extract. The skin reaction may, in this event, be a composite of reactions to two or more substances which would entirely invalidate it as a means of determining the amount of *active substance* in the extract. Again, it must be kept in mind that both the scratch method and the intradermal injection are in use and that reactions obtained by the two methods may not be quantitatively comparable.

To avoid these difficulties this investigation was carried out entirely upon myself as I am a typical ragweed-sensitive individual. Believing that the typical nasal reaction to pollen might be used as a satisfactory standard for measuring activity of extracts, these were introduced into the nose in increasing amounts until sneezing was produced. It was found that when this was done in the part of the year when there was no ragweed pollination and when there was no nasal irritation from other causes, the minimal amount of a given extract which would produce sneezing was exceedingly constant. Sneezing would begin within five minutes and, if 0.1 mil of a given solution was required on a given day the same amount would produce the same reaction on any other day and less than this amount would not elicit any reaction on any day. Because of its dependability and because it made it possible to get away from the questionable skin reaction, the intranasal reaction was adopted as the index of activity.

The pollen extracts used included ragweed extracts from each of the manufacturers marketing these products and two extracts made by myself. The two latter were made by the method proposed by Coca.[2]

There are three methods of standardization now in use: (1) A given amount of pollen is extracted with a given volume of extracting fluid and the resulting extract designated as a given dilution of whole pollen or as containing so many pollen units. (2) The extract is Kjeldahled and the nitrogen content used as the basis of calculation. (3) By complement fixation.

The fallacies inherent in the first method are apparent. First, if given amounts of pollen are extracted with fluids of different composition, the solubility of the active substance in the different fluids may produce extracts of widely differing activity and these extracts would not be comparable. Secondly, extracts made by the same method may vary in content of active substance. Thus, the two extracts made by myself, using the same extracting fluid and technic throughout with different batches of pollen (but secured from the same source) yielded solutions one of which was 62½ times as potent as the other.

Standardization by nitrogen content has for some time been considered unsatisfactory because of the belief that the antigenic nitrogen compounds form only a part of the total protein present and the nitrogen determination makes no distinction between antigenically active and inert protein. Previous investigators[3] have concluded that this is an inadequate method, while Grove and Coca[4] have recently stated that extracts digested with trypsin and dialyzed are still active as formerly.

We have made nitrogen determinations on eight ragweed extracts. Table I shows the nitrogen content as compared with the minimal amounts required to produce the intranasal and intradermal reactions.

It is readily seen that the nitrogen content has no quantitative relationship to the activity of the solution. Whereas the largest nitrogen content is only 18 times the smallest, the largest amount required to produce sneezing is 2.500 times the smallest. Also the solution containing the most nitrogen is only one-half as active as the one containing the least.

If it be insisted that the skin reaction be taken as the index of activity it is again seen that there is no relationship. The solution with the least nitrogen content is eight times as active as that containing the most, while the dilution of the most active extract producing a reaction is 6,400 times as great as the dilution of the least active producing the same reaction. Extracts C1 and C2. made in the same way, show a wide variation in nitrogen content and activity both in nose and skin, C2 containing little more than one-half the amount of nitrogen in C1 but reacting 62½ times as well in the nose and 128 times as well intradermally.

TABLE I

EXTRACT	MG. N. PER MIL	INTRANASAL UNIT	INTRADERMAL UNIT
A1	1.26	.1 mil	1-1,000
A2	.28	.1	1-4,000
C1	.98	.005	1-20,000
C2	.56	.00008	1-2,560,000
L	.42	.005	1-8,000
M	.07	.05	1-8,000
P	.14	.2	1-400
S	.70	.00025	1-1,280,000

Table I also illustrates the relative parallelism of the intranasal and intradermal reactions; that is, those most active intranasally are also most active in the skin and vice versa. This parallelism in one who has had treatment with pollen extracts indicates that the possibility previously mentioned of reactions in the treated patient to protein which may have no relation to the true reaction of the hay-fever patient, is probably not of any moment.

We were able to repeat the work of Grove and Coca[4] and, by digestion with trypsin and dialysis against distilled water, secured an extract which no longer gave a biuret reaction. This digested extract reacted intranasally and intradermally in the same dilutions as it did prior to digestion.

The method of standardization by complement fixation first advocated by Clock[5] has been believed to offer a means of determining the antigenic protein as distinguished from that which is antigenically inert. It is possible to inject animals with pollen extracts and produce antibodies against them. We have found, too, that the serum of ragweed-sensitive persons contains a substance which binds complement in the presence of ragweed pollen. In tests done using my serum it was found that 0.002 mil gave complete fixation of complement. Tests were then done using 0.2 mil of a 1-10 dilution (10 units) of serum and the results are shown in Table II.

TABLE II

EXTRACT	MG. N. PER MIL	UNIT FOR COMP. FIX.	INTRANASAL UNIT
A[2]	.28	.02 mil	.1 mil
C[1]	.98	anticomp.	.005
C[2]	.56	anticomp.	.00008
L	.42	.05	.005
M	.07	.2	.05
P	.14	.02	.2
S	.70	.002	.00025

It will be seen that the two extracts made by Coca's method were anticomplementary and complement fixation as a method of standardization was, with them, impossible. The extract with the least nitrogen content was the weakest antigenically while that with the highest amount of nitrogen was the most potent. Between these extremes the parallelism was not regular. On the other hand, a comparison of the binding power with the intranasal activity shows only a general approximation. Extract P which has ten times the antigenic value of Extract M is only one-fourth as active in the nose.

Complement-fixation tests done on the sera of treated and untreated hay-fever cases lead us to believe that the complement-binding substance is produced as a response to the introduction of some one or more of the pollen proteins and not necessarily to the active substance itself. Three individuals who received from 20 to 30 injections of pollen extract during the past season were capable of binding complement with 0.002, 0.002 and 0.001 mil of serum, respectively, while of five ragweed-sensitive individuals who have never had any treatment, the amounts of serum required varied from 0.1 to 0.3 mil. This small number will not permit of any hard and fast conclusions but we are inclined to believe that the complement-binding substance is found in the

serum of the untreated as a result of absorption of the protein through the mucosa of the upper respiratory tract while the increased amount in the sera of treated persons is due to the repeated injection of the protein.

If the foregoing assumption may be questioned we feel that we have more dependable data in the results of tests done with digested extracts. Extract L whose antigenic unit was 0.05 mil was antigenically inert after digestion, although its activity intranasally and intradermally was unchanged. Guinea pigs which had been injected with Extract C2·(which was anticomplementary and could not be used for fixation tests) responded with the production of an antibody which fixed complement in the presence of other ragweed antigens. These sera gave no binding with the digested extracts. Extracts L and C2 were mixed with equal parts of my serum and injected intradermally into the forearm of a nonsensitive individual.[6] Typical reactions were obtained. These extracts, after digestion, although no longer capable of serving as antigens in complement-fixation tests, produced the same reaction, quantitatively, in the arm of this nonsensitive individual.

Since extracts which have been digested and can no longer serve as antigen in complement-fixation tests, are as active intranasally and intradermally as before, we believe the conclusion is justified that complement fixation as applied to pollen extracts is only another method of determining the protein in the extract and, while it may serve to distinguish some proteins from others, it is, as a means of estimating the amount of active substance, entirely valueless.

THE NATURE OF THE ACTIVE SUBSTANCE

The work presented in the foregoing statement warrants the conclusion, we believe, that the active substance in pollen is not protein. Grove and Coca[4] suggested that it resembled an enzyme. We have shaken ragweed extract four hours in a mechanical shaker and found its activity unchanged. It has been frozen solid for six days and lost none of its activity. Boiling for ten minutes and heating it in the autoclave at ten pounds for ten minutes did not affect it. The evidence does not support the assumption that the active substance is an enzyme.

The work done by Heidelberger and Avery[7] in which they found in pneumococcus substance what they believe to be a polysaccharide capable of producing precipitates with immune serum, led us to attempt to correlate the activity of pollen extracts with their glucoside content. This work has only been begun but, thus far, we have found that the Molisch reaction does not parallel the activity of the extract and reduction of Fehling's solution does not seem to be increased after hydrolysis. This work is still in progress.

EXPERIMENTS BEARING ON ANAPHYLAXIS

In an attempt to produce active anaphylaxis in guinea pigs Extract C2 (containing 0.56 mg. nitrogen per mil) was injected intraperitoneally into six pigs whose weights were from 250 to 320 grams. Three pigs received a single injection of 2 mils for each 250 grams body weight. Three received one mil per 250 grams body weight and forty-eight hours later a second

injection of the same amount. Five days after the first injection one pig which had received the divided doses was found dead. Two days later one receiving a single large dose died. On the tenth day three were found dead and on the eleventh the last succumbed. No evidence of illness was noted except a slow but constant loss of weight, which, in no instance, exceeded 15 per cent. In each instance the animals were found dead without any opportunity to observe any agonal symptoms   Autopsies revealed no gross lesions. Since this was the extract which proved to be 62½ times as potent as the next most active extract, it was thought that death was probably due to excessive dosage.

Six pigs weighing from 250 to 350 grams received one mil of Extract C2 intraperitoneally and fourteen days later were given the same amount intracardiacally. No symptoms even remotely suggestive of anaphylaxis were observed. Three pigs received 5 mils each of my serum intraperitoneally and forty-eight hours later were given one mil Extract C2 into the heart. The pigs were apparently entirely unaffected. Two rabbits were injected intracardiacally with a freshly made mixture of 2 mils of Extract C2 and 5 mils of my serum. They showed no symptoms of any kind. Two rabbits received similar mixtures in the veins of the ear with no effect.

The pigs which had received the sensitizing and shocking doses of pollen without effect were bled and their serum separated. These sera, when mixed with Extract C2 and injected intradermally into the forearm of a nonsensitive individual, produced a typical reaction.

Although in this small series of animals we were unable to elicit any evidence of anaphylaxis, we would not conclude that anaphylactic reactions cannot be secured with pollen extracts. Alexander,[8] working with extracts of rye pollen, reported typical reactions. We believe that the difference in results is due to the fact that he was working with a solution containing 2.01 mg. of nitrogen per mil while ours contained only 0.56 mg. We believe that anaphylactic reactions may be secured by the use of extracts rich in protein and poor in active substance but that, like complement fixation, these reactions are reactions to the protein of the extract and have no relation to the active substance.

SUMMARY

1. The reaction of the nasal mucosa of a ragweed-sensitive individual may serve as a reliable criterion for the determination of the amount of active substance in ragweed pollen extracts.

2. None of the methods of standardization of pollen extracts now in general use are of value.

3. Solutions made by the same technic may differ widely in content of active substance.

4. The active substance is not protein.

5. The complement-binding substance is apparently increased by treatment.

6. Complement fixation, as applied to pollen standardization, is only another method of estimating certain protein constituents of pollen.

7. No evidence of sensitization of skin to protein, as distinguished from active substance, in treated cases was found.

8. Experimental evidence suggests that hay-fever is not an anaphylactic phenomenon.

For much technical assistance I am indebted to Misses Annette Black and Dorothy Branch and Mr. R. M. Smith.

REFERENCES

[1]Scheppegrell: Hay-Fever and Asthma, Phila., 1922, 124.
[2]Coca, Arthur F.: Jour. Immunol., 1922, vii, 163.
[3]Vander Veer, Albert: Jour. Immunol., 1922, vii, 113.
 Bernton, Harry S.: Jour. Am. Med. Assn., 1923, lxxx, 1301.
[4]Grove, Ella F. and Coca, Arthur F.: Proc. Soc. Ex. Biol. and Med., 1923, xxi, 48.
[5]Clock, R. O.: Jour. Infect. Dis., 1918, xxii, 80.
[6]Coca, Arthur F. and Grove, Ella F.: Proc. Soc. Ex. Biol. and Med., 1923, xxi, 49.
[7]Heidelberger, M., and Avery, O. T.: Jour. Ex. Med., 1923, xxxviii, 73.
[8]Alexander, M. E.: Jour. Immunol., 1923, viii, 457.

IS PATHOLOGIC NOMENCLATURE EITHER SCIENTIFIC OR SENSIBLE?*

By O. J. WEST,† M.D., SEATTLE, WASH.

RECENTLY, before our local Medical Society, an orthopedic surgeon read a very excellent paper on "Bone Tumors."

In the course of this paper he presented a classification of these growths, entirely of his own devising, extenuating this by explaining that recent textbooks differ so widely in their terminology that the more one reads, the more he becomes confused.

I, being a pathologist, even I may say, being an old-fashioned pathologist; a pathologist of the period far back in the good old days when the embryonic layers were epi-, meso-, and hypo-blast, the epiblast developing into pavement epithelium and nervous tissue, the mesoblast giving rise to the framework structures of the body, the hypoblast to glandular epithelium, each layer attending strictly to its own work, never encroaching on the field of the others; when malignancy of epithelium was always carcinoma and malignancy of mesoblastic structures always sarcoma. As I have said, being an old-fashioned pathologist trained in this atmosphere of exactness, I would have resented my colleague's intimation that pathologic terminology is lax and erratic, had I not recently made various book reviews for our local medical journal.

These reviews were of textbooks on pathology and tumor diagnosis and, perusing them, one after another, one needs must be impressed with the faulty and unscientific nomenclature in vogue.

For the purpose of specific data, I visited the medical library and briefly sketched through several of these, presumably, authoritative works. In them I found no less than 12 *malignant* growths described under names which in no way indicated their malignancy, nor whether of epithelial or connective tissue origin—misnomers, such as cylindroma and psammoma—a tumor of cylinders and a tumor of sand! I ask you, is that scientific or even common sense? Are the cylinders and the sand of such importance that they should command the terminology, to exclusion of the malignancy resident within these growths?

Nor does the perniciousness end with this. Just so surely as a tumor is named from some *characteristic*—not the tissue of its origin, just that surely will inaccuracy and confusion arise.

This is exemplified in both the misnomers I have mentioned.

In Prof. Ewing's work on, "Neoplastic Diseases," page 294, he describes cylindroma among the endotheliomata, on page 705, he says: "Under the term cylindroma has been described a rather frequent form of adenocarcinoma of the salivary glands"

Wood's revision of Delafield and Prudden says: "Some of these growths are carcinomata." What the others are he does not state, but, already, we have the same growth, an endothelioma, an adenocarcinoma, a carcinoma and so named that it indicates neither.

Psammoma fares no better. Ewing, page 296, classes it with the endotheliomata and on page 424, writing of psammoma of spinal dura says: "The structure presents * * * a cellular and vascular form of sarcoma." Other authoritative works say they are composed of either fibrous, sarcomatous, or endotheliomatous tissue. In other words a psammoma may be a benign fibroma, a malignant sarcoma or a semimalignant endothelioma.

Again, is there either science or sense in such terminology? Striving for clarity only turbidity has resulted. Nor is this roiling of the pathologic waters necessary. If this sand is truly of importance it can be incorporated into the nomenclature without outraging pathologic tenets. Call it a psammomatous-fibroma, or sarcoma, or endothelioma, as the case may be.

We also find various neoplasms named from the *organ* wherein they originate, such as thymoma, hepatoma, hypernephroma. To justify such terminology these should be an autonomous growth of *all* the tissues of these organs in orderly arrangement. Instead, thymoma is really a lymphosarcoma, hepatoma is adenoma or carcinoma and hypernephroma, a tumor of *adrenal* tissue, sometimes orderly, often malignant.

Is this scientific?

Even in the nomenclature of pathologic tissue-changes, confusion and inaccuracy appear in ever-increasing amounts.

Seemingly this arises mainly from efforts diagnostically to connect the histologic findings and the clinical symptoms. In other words, having certain symptoms, there always must appear certain characteristic histologic changes.

I do not believe that any amount of study *ever* will accomplish this, for the insurmountable reason that different individuals will not react uni-

formly to the same tissue change, more than they will react in the same way to such patent things as an infection process, or drugs, or even light and heat.

Who among you would stake his pathologic reputation that a case showing frank fetal adenoma of the thyroid would have had thyrotoxic symptoms?

The pathologic changes are what they are, let the symptoms be what they will, and so soon as we permit the clinician, whether internist or surgeon, to tamper with our terminology, just that soon will confusion and obscurity obtain.

Some surgeons refuse to consider a growth malignant unless it customarily metastasizes, be the cells never so anaplastic and atypical. The influence of this idea already is found in our nomenclature, and, should the pathologist accept it, further confusion will arise.

I believe that only by adhering strictly to structural histology will pathologic nomenclature be fitted to a science.

I am fully cognizant that some histologic carcinomata and sarcomata very rarely metastasize. Does that preclude them being designated as carcinomata and sarcomata? Is a nonmetastasizing carcinoma so *vast* an anomaly when compared with other disease-conditions as we know them!

Since it is recorded that man has died of the itch and has recovered from carcinomatosis, who would dare forecast with absolute certainty how this human body will react to a given condition or what will be the conduct of a given neoplasm that is implanted in that body! The only thing that may be absolutely certain about a tumor is its histologic structure, and science can be built only on certainties. For this reason I decry the tendency to dominate pathologic nomenclature by *characteristics* of tumors, or *clinical symptoms* of pathologic changes. Our terminology must be based upon *fundamentals* if we would hold it free from obscurity, and it is surprising to what few basic elements microscopic histology can be reduced if we hold fast only to proved facts and ignore theory and surmise.

I have no doubt many of you would be extremely skeptical were I to state that there are but two *general* types of tissues in this wonderful human body of ours, and yet I can assure you that it so closely approximates actuality that, for years, it has been of the greatest aid to me in pathologic study.

By "two general types" of tissue I mean; first, the tissue which *secretes* —the *functional* tissue of an organ, and second, the *framework* tissues that hold this functional portion in place.

Tersely, secreting tissue and framework tissue.

Concisely: epithelium, and the tissues of mesoblastic origin. Of the epithelium there are two varieties, but malignancy of *either* develops into carcinoma. Of the mesoblastic structures there are several, fibrous, muscle, bone, fat, cartilage, lymphoid tissue—but malignancy of each develops into sarcoma. Thus we have but two types of malignancy, carcinoma and sarcoma.

In apposition, from a single textbook I glean an impressive array of: acanthoma, adamantinoma, astrocytoma, chloroma, chorioma, cylindroma, folliculoma, hepatoma, hypernephroma, myeloma, psammoma, splenoma, thymoma, etc., yet all of these are really either carcinoma or sarcoma.

Are we being more exact in return for the increased burden pathologic literature must bear? We are not. What accuracy is there in terming a malignant growth a tumor of thorns, or stars, or chlorophyl, or follicles or sand?

While the pathologist may be trained to knowledge of the meaning and significance of these terms, it should be borne in mind that the field worker, the clinician, is the terminal of all our pathologic labor, and what is he to do with such?

It is all so needless! If a growth is a cancer, let it be so called, if it is sarcoma, so name it, and add to these any enlightening terminology desired, but let them be called carcinoma or sarcoma, first, last, and all the time.

Granted that they vary in clinical course and prognostic significance, is there any other pathologic state that does not, also, vary? Can you make pathology any more exact than are the diseases of which it is a study?

And does the naming of malignant growths after thorns and cylinders and sand, aid in the matter?

Criticism which suggests no remedy is better left unsaid, and the object of this paper is to preface the suggestion that a committee be appointed to draft a sane and sensible outline of nomenclature to be followed by the members of this Society.

I take the liberty of voicing my own views, not that they may be adopted, but that they may stimulate discussion of this subject. Personally, I feel that this outline should be based upon the normal tissues wherein the pathologic changes occur and that all further refinements of descriptive terminology be compassed by means of qualification of these terms, that malignancy of epithelium be designated as carcinoma; malignancy of mesoblastic structures as sarcoma and that the basis of microscopic study of diseased organs be the two types of tissues of which all organs are composed, parenchyma and stroma.

Since these comprise the entire organ it follows that any and all changes must be in one, the other, or both, and we will have parenchymatous, interstitial or diffuse lesions. With this as basis of our terminology, fewer pathologic impossibilities will be present in our literature, such as the very prevalent term, "Parenchymatous Nephritis." If this term is intended to convey the meaning of inflamed parenchyma of the kidney, it is a pathologic impossibility. Having no blood vessels, the parenchyma *cannot* inflame. If there is inflammation the *stroma* must also be involved and we have a diffuse nephritis. If the parenchyma *alone* is involved the change must be degenerative and cannot be inflammatory, therefore a nephritis does not exist. Prof. Wood has suggested that these noninflammatory degenerative changes be termed nephroses, which, to me, seems excellent.

As microscopic histologists, what are we to do with the so-called "precancerous" changes?

Modern textbooks show microphotographs of breast tumors, where the gland acini are widely separated by fibrous tissue, enlarged to 20 times their normal dimension and densely packed with cells which are rapidly multiplying, as shown by the more embryonic character of the cells and pressuring elongated nuclei. (See Ewing's Neoplastic Diseases, 1st Ed., Fig. 184.) Yet

this is called a *precancerous* condition. Presumably, because the cells have not yet broken through the basement membrane and invaded the surrounding tissue.

Is it rational to suppose that the first movement of a malignant gland-cell will be to break through and invade the resistant fibrous stroma? Or is it more reasonable to assume that, as these cells multiply, the resultant cell-mass will follow a law of physics and first progress in the direction of least resistance, which is the *lumen* of the gland? When the lumen is packed to the density of the surrounding tissue, only then would these rapidly proliferating cells invade the stroma.

This cell proliferation in the direction of least resistance is one of the gross diagnostic points of those cancers which are freely open to observation, skin cancer.

At first these growths are elevated because the proliferating cell-mass finds it easier to extend *upward* toward the surface than *downward* through the dense fibrous tissue. Later this fibrous tissue gives way and the malignant area may be depressed, but, invariably, at the edge, where there is yet firm fibrous tissue, the growth is elevated, giving a diagnostic point of some value.

Frankly, I am suspicious of any gland which has filled its lumen with actively proliferating cells, because this is contrary to nature's normal way. When the epithelium of a normal gland proliferates, cell division is longitudinal and at right angles with the periphery of the gland so that it leaves the new and the old cells shoulder to shoulder so to speak. These cells are like a company of soldiers formed in a circle shoulder to shoulder and facing inward: when typical mitosis occurs it is as if one cleaved a soldier from crown to crotch, each half then developing to a complete individual; were this continuously repeated, the line would become more compact, if there were slight peripheral resistance the circle would enlarge and we would have the equivalent of a cystic gland; when peripheral resistance becomes sufficient the line would "buckle" inward, remaining, however, as a single line, and we have the equivalent of a cystic gland with papillomatous protrusion into the lumen. When atypical mitosis of malignancy occurs, cell division is at right angles to that just described, the new illustrative soldier would then stand *in front* of the old and we soon would have many concentric lines, eventuating in a solid mass—the densely packed gland of malignancy.

One who has observed with what reluctance normal gland-epithelium will begin atypical mitosis and pack the gland-lumen with rapidly multiplying cells surely must attach grave significance to such findings.

We must suppose that such growths are called "precancerous" because they are not yet invasive or metastasizing, but to me, this is no more rational (following the old idea that smallpox is not infectious until the period of eruption) than to say that a case of smallpox is not smallpox but a "prevariolar" state, until the stage of eruption or infectiousness.

I do not believe the condition described is "precancerous." I think it is already cancerous, in all probability a preinvasive and premetastasizing stage of cancer but, nevertheless, carcinoma. I believe they should be desig-

nated as *early* carcinoma, but I also believe our reports should further state that the condition is probably as yet neither invasive nor metastasizing, in order that the patient may be subjected to only the necessary degree of mutilation.

In conclusion let me say that this paper was written because of the numerous criticisms of pathologic nomenclature which have been expressed in my presence by active workers in the medical field; and because I believe it lies within the province and influence of this Society to give a trend that is both scientific and sensible to the terminology used by its members.

Announcement

The Ninth Annual Clinical Session of the American Congress on Internal Medicine will be held in Washington, D. C., March 9-14, 1925

Washington clinicians and investigators of attainment will devote the entire session to amphitheatre and group clinics, ward "rounds," laboratory conferences, lectures, demonstrations of special apparatus and methods, and the exhibition of unusual scientific collections. Civilian and governmental services are united in the aim to make the week useful and memorable.

Practitioners and laboratory workers interested in the progress of scientific, clinical and research medicine are invited to take advantage of the opportunities afforded by this session.

Address enquiries to the Secretary-General.

Wm. Gerry Morgan, Pres.
Washington, D. C.

Frank Smithies, Sec'y-Gen'l.,
1002 N. Dearborn St.,
Chicago, Ill.

THE EFFECT OF VARIOUS ANESTHETICS UPON THE STRENGTH OF UTERINE CONTRACTIONS

By M. Pierce Rucker, M.D., Richmond, Va.

WHEN a hollow viscus contracts in an energetic manner pain is a result. The uterus is no exception to this rule. In no other organ, however, do we associate the idea of force with such cramps. The intestines or the gall bladder may contract as painfully as the uterus, but we do not get the impression of forcefulness that we do in the presence of labor pains. From time immemorial force and pain have been so inseparably associated with parturitional contractions that we unconsciously use the two terms interchangeably.

The force of labor pains has been variously estimated at from 4 to 577 pounds.[1] This wide variation was due to the different methods used, such as measuring the force required to rupture the amniotic membranes outside the body, the pull necessary to deliver the infant with forceps, etc. In 1866 an instrument designed by Carcassonne[2] to measure the contractions of the uterus in parturition was presented at a meeting of the French National Academy of Medicine. There was no description of the instrument which was called the "metrodynamomètre," nor can I find any record of the work done with the instrument. When twelve years later Poulet[3] presented his instrument, which he called the "tocograph," before the Surgical Society of Paris a committee composed of MM. Guéniot, Lucas-Championnière and Polaillon was appointed to look into the merits of the instrument. Polaillon gives a description of the instrument in his report[4] and states that the only other similar work is that of "Schartz of Rastok." The tocograph consists essentially of two mercury manometers, one connected to a rubber bulb that is placed within the uterus and the other to a similar bulb that is placed in the rectum above the fetal head. The manometers were provided with floats and writing points, and in this way records were made upon Ludwig's kymograph of the action of the uterus and of the abdominal muscles. At the conclusion of his report M. Polaillon moved (and it was adopted):

1. "De remercier M. Poulet de son travail.

2. "De déposer sa mémoire aux archives.

3. "De publir, dans nos mémoires, la figure indiquant les courbes des pressions utérines et musculaires."

Nevertheless in the published memoirs there are no uterine tracings illlustrated.

In 1872 Schatz[5] published his first work upon uterine contractions, and his curves and figures are still referred to in American obstetrical literature.

Schatz found an intrauterine pressure of 20 mm. of Hg. between pains, and of from 80 to 250 mm. of Hg. at the acme of contractions. Polaillon's[6] work in 1880 is in some respects even more interesting, but has attracted little notice in this country. He used an intrauterine rubber bulb and a mercury manometer for measuring the force of uterine contractions as did Schatz and Poulet, but for recording the contractions he used a Marey tambour. He
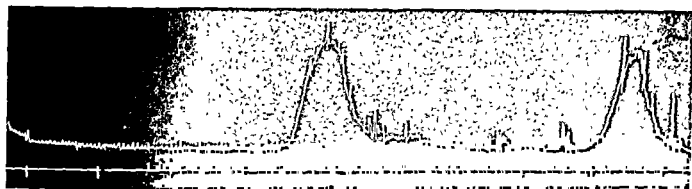
Fig. 1.—Internal hysterogram showing first stage pains with small reflex contractions of the abdominal muscles at the top of the contractions and respiratory movements between contractions. The timer in this and all subsequent figures marks minutes
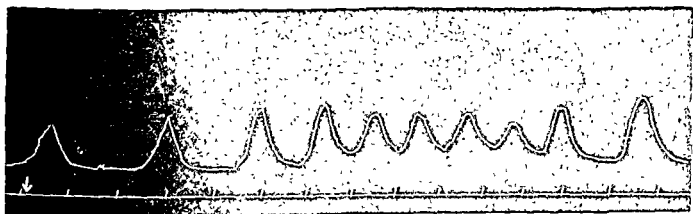
Fig. 2.—Internal hysterogram from a patient whose labor was induced prematurely and who was given 2 minims at the point indicated by the arrow. Note that there was very little effect, but that the uterus did not relax completely for nine minutes.

Fig. 3.—This patient was at term and was given 2 minims of pituitrin at the point indicated just at the end of the second contraction in the record In this instance there was a marked increase in intrauterine pressure that did not subside for 28 minutes.

divides the accessory uterine movements into intrinsic and extrinsic. Among the latter may be mentioned respiration and sighing, crying, sneezing, muscular efforts in moving the head or the body, straining, micturition, defecation, pulsations of the maternal arteries, and pressure of the hand on the abdomen. The intrinsic accessory movements are the fetal movements, which

are very irregular. Polaillon marked on the curves of uterine contractions the exact point at which the patient felt pain and when she ceased to feel the pain. He found that this occurred constantly when the curve reached a height of 12.25 mm. of Hg. and ceased when it fell below 10.45 mm. of Hg. In a fatal case he weighed the uterus, and by calculating the amount of work that had been done from his records, estimated that a gram of uterine muscle was capable of putting forth 178 grams of energy which is small compared to the figures (1087 grams) given for striated muscle. In 1898 Hensen[11] investigated the action of morphine and ether upon uterine contractions, using Schatz's apparatus. Schatz, Poulet and Polaillon all used small rubber bulbs of from 70 to 80 c.c. capacity, that were placed within the uterus between
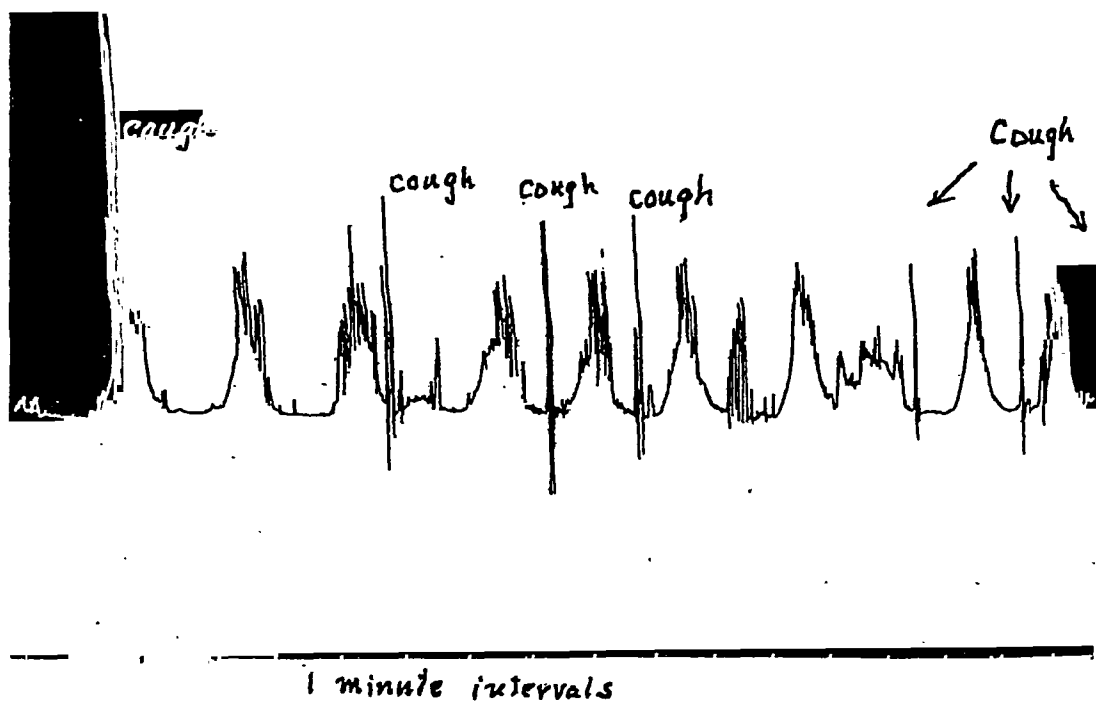
Fig. 4.—Internal hysterogram showing first stage pains with superimposed involuntary contractions of the abdominal muscles. Between the pains are shown the effects of coughing (sharp straight lines) and of respiration, (the tiny waves of 1 or 2 mm. in height).

the membranes and the uterine wall for the purpose of registering the variations in intrauterine pressure. Several years ago, it occurred to me[7, 8] that I might in the same manner make use of a Voorhees bag that had been placed within the cervix, without detriment to the patient. The stem of the bag was connected with a mercury manometer by means of a thick wall rubber tube. The distal arm of the manometer was fitted with a float that carried a writing point.

Fig. 1 is from a tracing of normal first stage contractions obtained in this manner. It is very similar to Schatz's and Polaillon's. It shows an intrauterine pressure of 10 mm. of Hg., while at the height of the contraction the pressure reaches 66 mm. of Hg. You will notice that the respiratory

movements cause a variation of several mm. of Hg. in intrauterine pressure. Figs. 2 and 3 show the importance of accurately measuring the variations in intrauterine pressure when studying uterine contractions. It is commonly stated that pituitary extract augments normal uterine contractions, but does not cause tetanic contractions. What happened in these two instances, however, was that after doses of two minims of pituitrin the uterus went into incomplete tetanus with superimposed waves of contraction, which to the palpating hand on the patient's abdomen would feel like normal contractions with periods of relaxation in between. Fig 4 shows the effect of coughing and Fig. 5 the effect of vomiting and of straining in an effort to void. It is very evident that almost any use of the abdominal muscles is attended by a greater intrauterine pressure than is a contraction of the uterus, but for a much shorter time.

This method of internal hysterography is especially well suited to the study of the effect of various anesthetics upon the strength and frequency
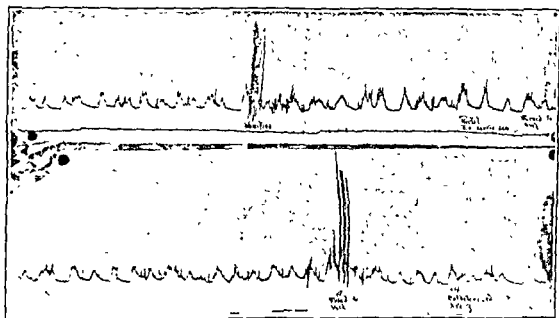
Fig. 5.—Internal hysterogram showing the effect of efforts at voiding and also of vomiting.

of uterine contractions. Chloroform, since the discovery of its anesthetic properties by Sir James Y. Simpson, has been considered, certainly until very recently, the obstetric anesthesia *par excellence*. Sir James Y. Simpson considered it superior to ether for the following reasons:

1. "A greatly less quantity of chloroform than of ether is requisite to produce the anesthetic effect; usually from 100 to 130 drops of chloroform only, being sufficient, and with some patients less. I have seen a strong person rendered completely insensible by six or seven inspirations of thirty drops of the liquid.

2. "Its action is much more rapid and complete, and generally more persistent. I have almost always seen from ten to twenty full inspirations suffice. Hence the time of the surgeon is saved and the preliminary stage of excitement, which pertains to all narcotizing agents, being curtailed, or indeed practically abolished, the patient has not the same degree or tendency to exhilaration and talking.
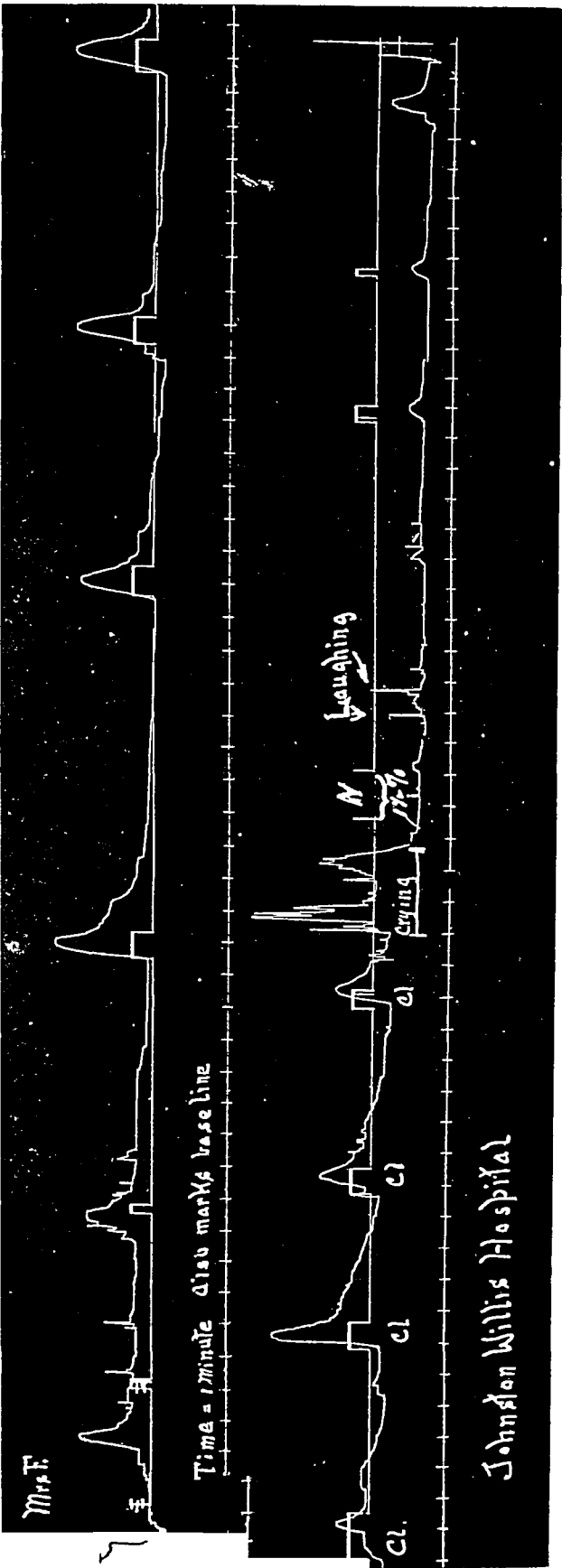
Fig. 6.—Internal hysterogram showing the effect of chloroform given *à la reine*. At the points marked "Cl" chloroform was given in sufficient quantities to somewhat ease patient's pains and to make her hysterical. One and one-half per cent novocaine (35 c.c.) was given sacrally at the place marked "N." The line between the timer and the writing point of the manometer (above it toward the end of the record) is made by an electric magnet that is controlled by push button in the patient's hand, so that the patient can indicate when she feels pain. Note the last contraction on the record is painless.

3. "Most of those who know from previous experience the sensations produced by ether inhalation and who have subsequently breathed the chloroform, have strongly declared the inhalation and influence of chloroform to be far more agreeable and pleasant than those of ether.

4. "I believe that considering the small quantity requisite as compared with ether the use of chloroform will be less expensive than that of ether.

5. "Its perfume is not unpleasant but the reverse, and the odor of it does not remain for any length of time obstinately attached to the clothes of the attendant, or exhaling in a disagreeable form from the lungs of the patient, as so generally happens with sulphuric ether.
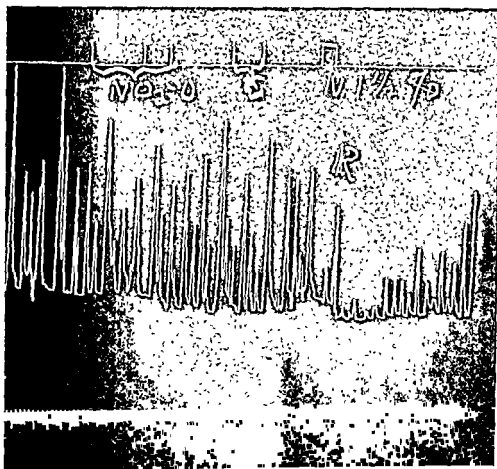
Fig. 7.—Internal hysterogram showing the effect of (1) nitrous oxide-oxygen; (2) ether given *à la reine*. In neither instance was there enough anesthetic given to either relax the patient or to render her unconscious. Note that there was some effect upon the strength and frequency of contractions after the nitrous oxide-oxygen was begun and a step-like rise as soon as it was stopped. The effect of ether was more marked. Later the patient was given 35 c.c. of 1½ per cent novocaine solution with 5 minims of adrenalin solution into the sacral canal. The pains stopped entirely for 11 minutes and when they again started were only one-third their former force. "R" indicates a rectal examination.

6. "Being required in much less quantity, it is much more portable and transmissible than sulphuric ether.

7. "No special kind of inhaler or instrument is necessary for its exhibition."

Even today there is much difference of opinion among obstetricians as to the effect of chloroform upon uterine contractions. Vignes[10] says that this is in part due to the little attention that has been paid to the "temperament" of the anesthetized. There is a great difference between a "country" uterus and a "city" uterus, between the uterus encountered in the clinic and the one met in private practice. The method and time of

Fig. 8.—Internal hysterogram showing the effect of ethylene administered *à la reine*. The contractions are stronger and last longer. The patient was relieved of sensation of pain.

administration are other important factors. In exceptional cases chloroform can be given *a la reine* for long periods of time without materially influencing the progress of labor. Usually, however, if given too early it seriously interferes with the progress. Fig. 6 shows what is probably the usual effect. There is a diminution in the height of, and a lengthening of the interval between contractions. Fig. 7 shows almost as marked an effect from the administration of ether in the same manner, i.e., *a la reine*. This is in accord with Hensen's[11] findings.

It is quite commonly believed that nitrous oxide-oxygen not only does not diminish the force of uterine contractions, but may even increase it. Danforth and Davis[12] in a recent article say "nitrous oxide is the one thing yet thoroughly tested which will relieve the pain of labor without at the same time diminishing the force and frequency of the uterine contractions, and which may be used for periods up to several hours without appreciable loss in the
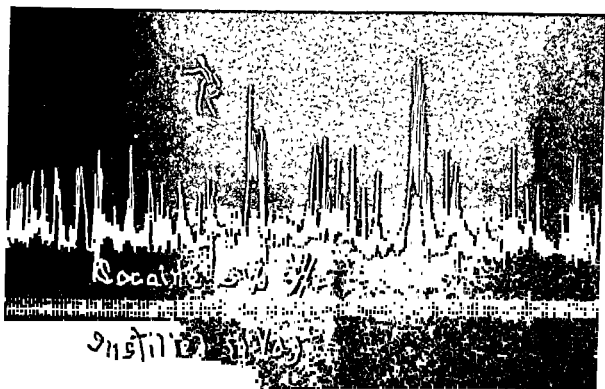
Fig. 9.—Internal hysterogram showing the effect of the application of 5 per cent cocaine solution to the cervix.

force of the uterine contractions." That this is not invariably the case can be seen in Fig. 7. Here there was diminution in both force and frequency of contractions so long as the gas was given, and it was given intermittently at each pain. As soon as the gas was stopped there was a very prompt increase in the strength and the duration of the pains.

Fig. 8 shows the effect of ethylene. When the gas was started at the point indicated the patient seemed to suffer more and became very restless. There was some mental confusion as is shown in the record by her signaling that she had a pain when the uterus was not contracting. The uterine contractions became stronger and lasted longer and were augmented by contractions of the abdominal muscles as indicated by the sharp lines on the summit of the uterine waves. When asked about it afterwards the patient said the gas gave her the greatest relief, that while she knew that she was having pains she did not experience the pain.

Cocaine applied to the cervix has been of distinct value to me in introducing bags, especially in primiparae. Not only does it cause some relaxation and deadens sensation, but it also softens and even dilates the cervix. Fig. 9 is a hysterogram from a patient into whose vagina 15 minims of a 5 per cent cocaine in 1-1000 acriflavine solution was instilled after the bag was placed. Unfortunately, the patient was restless and the record shows a great deal of movement of the abdominal muscles. It is very evident, however, that after the cocaine was applied the uterine contractions were less frequent.
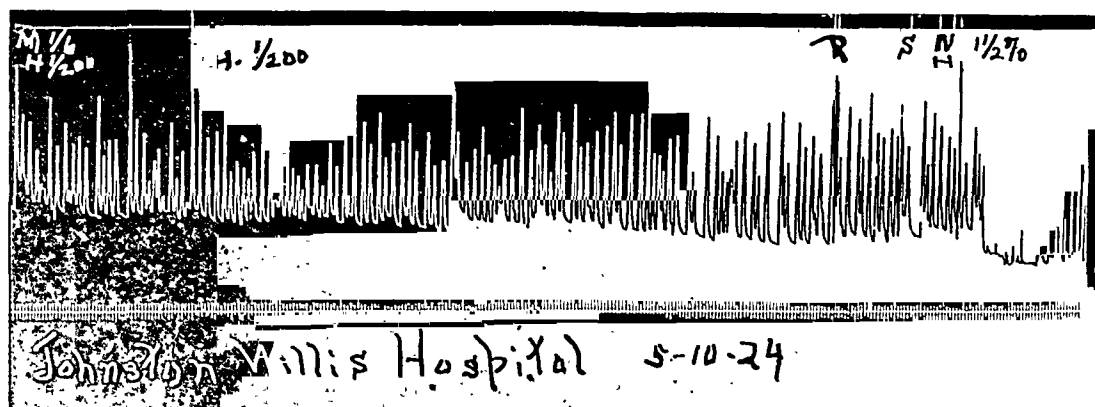
Fig. 10.—Internal hysterogram showing the ordinary effect of sacral anesthesia with novocaine (with 5 m. of adrenalin). Ten minutes after the injection was completed all contractions ceased for a period of 22 minutes. The force of contractions then gradually increased until it became as great as it was before. There was however no pain.
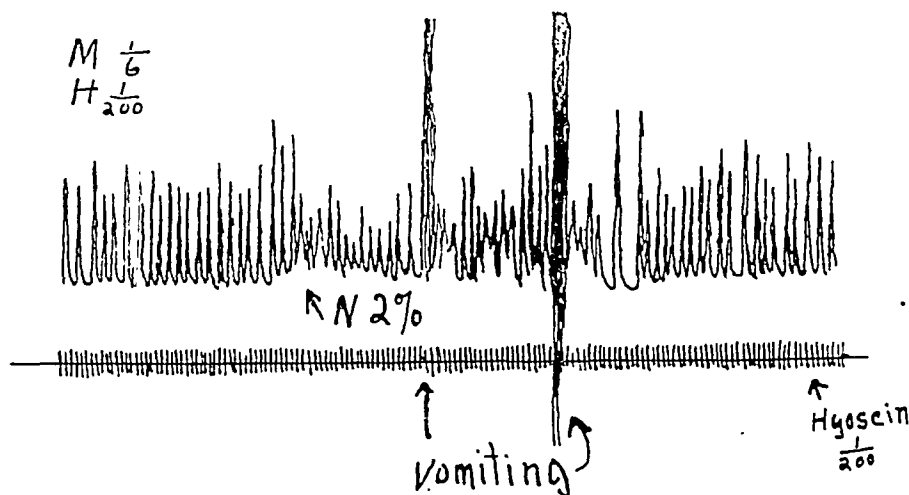
Fig. 11.—Internal hysterogram showing cessation of uterine contractions for an hour and fifteen minutes after sacral anesthesia with novocaine and adrenalin.

and of about one-half of their former height. This effect lasted for thirty-two minutes when the patient had a long hard contraction.

Novocaine given sacrally, has inconstant effects upon uterine contractions. According to Bonar and Meeker,[13] in the majority of their cases there was complete cessation of contractions within ten minutes after the injection was completed. "This diminution rarely lasted more than twenty minutes, after which the contractions gradually increased in frequency and duration until shortly the contractions proceeded normally." The majority of our

cases have conformed to this type and Fig. 10 is an excellent example. Here the contractions ceased in ten minutes and did not reappear for twenty-two minutes. In ten minutes more the contractions had regained their former force, but their duration was still less than it was before the novocaine was administered. The contractions were not accompanied by the sensation of pain. In some cases, however, there is a complete cessation of contractions as long as the effect of the novocaine lasts. Fig. 11 shows such a result. In only one of my cases was there an increase in the frequency of the contractions after the use of novocaine in this manner. In this patient (Fig. 12) the pains immediately increased and were fully as strong as formerly. Had I not obtained perfect relaxation and anesthesia, I would have thought
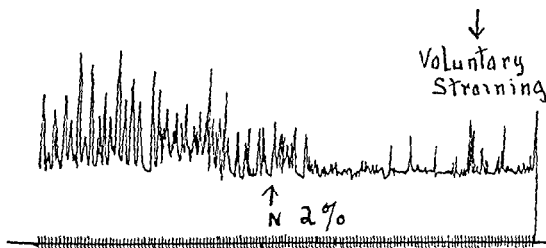
Fig. 12.—Internal hysterogram showing an unusual effect of novocaine given sacrally. Here the contractions were more frequent and were fully as strong. This patient was a primipara and was delivered by version. She had excellent relaxation and anesthesia.
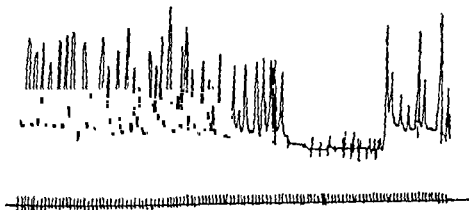
Fig. 13.—Internal hysterogram showing effect of puncture without giving any drug. In this case I was unable to locate the sacral hiatus with my needle. Nevertheless the contractions ceased for 28 minutes.

that I failed to give the novocaine into the sacral canal and was getting only an adrenal effect. The patient was extremely neurotic and I purposely did not give her any drugs of any kind. I simply applied a 5 per cent cocaine solution in 1-1000 acriflavine to the vulva and to the cervix before placing the bag, and when she reached the latter part of the first stage, I gave her novocaine sacrally. There was no sensation of pain after the novocaine was injected. She was delivered by version and there was extreme relaxation of the perineum and cervix.

Fig. 13 shows the effect that may follow a puncture in the region of the sacral hiatus. In this case, I was unable to get my needle into the hiatus

LABORATORY METHODS

UREA DETERMINATIONS IN BLOOD AND URINE*

By T. Addis, M.D., San Francisco, Calif.

THE methods for the determination of urea in urine and in blood which are given in this paper have been gradually developed by a number of workers in this laboratory during the past ten years. They are based on the procedures published by Marshall[1] in 1913. Some modifications were described by Addis and Watanabe[2] in 1916. Since then several important alterations have been made but for the last two or three years there has been no essential change. They are now more accurate and considerably more convenient than before and they may be useful to those who are interested in the study of Bright's disease because they are particularly designed to meet the conditions met with in clinical investigation. But it should be remembered that they are recommended only for the determination of urea in urine and in whole blood kept from coagulating by potassium oxalate or sodium fluorid. In spite of a great deal of investigation we have been unable as yet to explain the discrepancies which are found when parallel determinations on whole blood, plasma and blood and plasma filtrates are compared. As a matter of fact very little is known in regard to the urease method. The mechanism of the decomposition of urea by urease is not yet decided[3] and even such a fundamental matter as the structure of the urea molecule is still uncertain.[4] It is well to recognize, therefore, that all urease methods are more or less empirical and that accordingly they demand a close observance of those apparently arbitrary requirements which experience has shown to be necessary.

SOLUTIONS

1. *Urease.*—This solution is freshly prepared each day. Whole jack beans are ground to a fine powder in an "Arcade" mill. The beans should not be more than one year old.† Ten grams of the powder is shaken up in 100 c.c. of water and incubated at 37° C. for thirty minutes. The filtrate is called urease.

2. *Inactivated Urease.*—Five c.c. of 1 per cent mercuric chloride solution is mixed with 45 c.c. of urease.

3. *Buffer Mixture.*—Eighteen liters of this solution are made at one time from concentrated stock solutions. One hundred and eighty c.c. of a solu-

tion containing 1.4 per cent sodium pyrophosphate and 0.2 per cent glacial phosphoric acid, and ninety c.c. of 0.1 N NaOH, are measured out in graduates and washed into an ordinary 19-liter distilled water bottle. Distilled water is run in, forty-five c.c. of 0.2 per cent rosolic acid dissolved in 70 per cent alcohol are added, and the solution is brought to the 18-liter mark and mixed. Toluol may be added to prevent the growth of moulds.

4. *Standard Urea Solution.*—A good grade urea is dissolved to saturation in hot alcohol. After cooling, the urea crystals are collected on a filter, redissolved and recrystallized as before, filtered and dried. A solution containing 0.3 per cent urea in water is prepared. It should be remembered that this solution may undergo bacterial decomposition even in the presence of toluol, but urea can be kept indefinitely in solution if it is dissolved in 0.01 N HCl. It is best, therefore, to make a 3 per cent solution in 0.01 N HCl, and to prepare a fresh 0.3 per cent solution by diluting 100 c.c. of this solution and 100 c.c. 0.01 N NaOH to 1000 c.c. with water.

5. *Barium Hydrate.*—The theoretical amount of barium hydrate required to make 18 liters of 0.01 N, is weighed and transferred to a 19-liter bottle. Boiled distilled water is added to the 18-liter mark. The exact normality is determined by titration with potassium acid phthalate as described by Dodge.[5] The phthalate can now be obtained in a very pure form for use as a standard in electrometric determinations of H-ion concentration.

6. *Hydrochloric Acid.*—An approximately 0.1 N solution is made by transferring the theoretical volume of concentrated hydrochloric acid from a graduate into a 19-liter bottle and filling to the 18-liter mark with distilled water. The concentration of the concentrated hydrochloric acid used can be read off in a table[6] from the specific gravity given on the label. The actual normality of the solution obtained in this manner is then carefully determined by repeated titrations of heated samples with a known solution of barium hydrate, using phenolphthalein as an indicator. No attempt is made to get an exactly 0.1 N solution.

7. *Saturated Potassium Carbonate.*—The C. P. brands of potassium carbonate often contain ammonia and are expensive. We buy U. S. P. potassium carbonate in 100 lb. lots.*, It is sometimes so dirty that it has to be filtered but we have never found ammonia. Saturated solutions are made in a series of vessels which are not so large that they cannot be easily handled and the contents are filtered into an 18-liter distilled water bottle. It is convenient to provide this large stock bottle with a syphon arrangement, and to draw off enough for each day's work into a small bottle.

THE METHOD FOR URINE

The optimal conditions are met when the urine contains from 0.3 per cent to 0.9 per cent urea. Urine obtained after diuresis from urea and water can be used directly, but specimens obtained in tests designed to test the urea concentrating capacity of the kidney should be diluted three to five times with water.

*Powers Weightman Rosengarten Co

One hundred c.c. of the buffer mixture is run from an automatic measuring device into each of three 250 c.c. capacity Erlenmeyer flasks. Five c.c. of urine is pipetted into each flask. If the contents of the flasks are not still pink after the addition of the urine the P_H is so far to the acid side that the activity of the urease may be inhibited, and 0.1 N NaOH is then added in equal amount to each flask until the pink color returns. To the first flask, 2 c.c. of inactivated urease is now added; to the second, 2 c.c. of urease and 5 c.c. of 0.3 per cent urea; and to the third, 2 c.c. of urease.

The flasks are allowed to stand at room temperature for forty-five minutes. In this laboratory the temperature varies very little from 20° C. but in hot climates a shorter time might be desirable, for after more than two hours at 20° C. there may be a slight loss of ammonia by diffusion from the open flasks.

At the end of the period of incubation two drops of 0.5 per cent methyl orange are added. The first flask is then titrated with the known hydrochloric acid solution (approximately 0.1 N). As the contents become neutral, the pink color due to the presence of rosolic acid disappears; as more acid is added the solution becomes cloudy on account of the precipitation of a protein in the urease and still later a pink tinge appears which is due to the methyl orange. The particular shade of pink taken as the end-point is immaterial but whatever is chosen becomes the end-point for the two other flasks which are titrated to the same tinge of color. The titration value of the urease alone is obtained by adding 2 c.c. to a flask containing 100 c.c. of water with methyl orange and rosolic acid which has been brought to the pink color chosen for the end-point, and the amount of acid to bring back this color is noted. The same procedure is used to find the value of the inactivated urease.

When 3 mg. of urea are completely decomposed the ammonia produced neutralizes 1 c.c. of 0.1 N acid. The second and third flasks were alike except that 15 mg. of urea (5 c.c. of 0.3 per cent urea solution) were added to the second. The second flask should therefore require 5 c.c. of 0.1 N acid more than the third. If it does, we are assured of the adequacy of the urease and also obtain a duplicate determination of the urea in the urine. When the titration figure for the inactive urease is subtracted from the number of c.c. of acid required for the first flask, we obtain the alkali value of urine in which no ammonia has been produced from urea. This urine value plus the urease value when substracted from the value for the third flask, gives the number of c.c. of acid neutralized by ammonia derived from the urea in 5 c.c. of urine. If the acid used is exactly 0.1 N the number of c.c. multiplied by three will give the amount of urea in 5 c.c. of urine in milligrams. The actual calculations are, however, carried out with the help of a slide rule because the acid is practically never exactly 0.1 N and errors are apt to be made when several figures have to be multiplied. The amount of urea in the whole specimen of urine is then obtained by multiplying by the volume in c.c. divided by five.

THE METHOD FOR BLOOD

The end of the piston of a dry 5 c.c syringe is smeared with vaseline and touched to finely powdered potassium oxalate or sodium fluorid so that a little sticks to the vaseline. The needles are sterilized in paraffin oil. About 5 c.c. of blood is drawn from a vein, the needle is detached, the piston removed, the nozzle closed with the forefinger of the left hand and 2 c.c. of the blood pipetted directly from the barrel into a "blood tube." We calibrate bulb pipettes to contain, not to deliver, for the viscosity of blood may vary so much that the delivery amounts are inconstant   The pipettes must therefore be washed free from all traces of blood. For this purpose a little porcelain crucible holding 6 c c. is filled with a solution prepared by diluting 20 c.c. of a mixture of 1.4 per cent sodium pyrophosphate and 0.2 per cent glacial phosphoric acid to 1000 c c with water, and this 6 c.c. amount is used for the washing of the pipette   Another 2 c.c volume of blood is then washed into another tube, and two other tubes are prepared to which only the washing fluid is added. Two c.c. of the urease used in the urine method are pipetted into each of the four tubes, the contents are mixed by shaking, the rubber corks are inserted and they are allowed to stand at room temperature for an hour.

Equal amounts (about 10 c.c.) of approximately 0.02 N hydrochloric acid, to which just enough methyl red (1 per cent solution in 70 per cent alcohol) has been added to give it a deep red color, are now placed in four acid tubes and boiled distilled water is run in until the level of fluid is 6 cm. high. When the period of incubation is ended, the blood and acid tubes are connected in series to a source of compressed air for aeration. The air passes first through a tube which contains 5 per cent sulphuric acid in order that it may be freed from all traces of ammonia, and is carried through the first blood tube to the first acid tube, and then to the second blood and acid tubes. Duplicate determinations are always run in tandem, because occasionally it is possible to demonstrate that a trace of ammonia escapes the acid in the first tube and is caught in the second. Since we may assume that this very small amount of ammonia is also lost from the second tube the titration of this tube will be more nearly correct and the first is used as a check. After all the connections have been made, the stoppers of the tubes containing the blood and urease or the urease alone are momentarily opened to allow of the introduction of a few drops of caprylic alcohol and of 16 c.c. of a saturated solution of potassium carbonate which is run in from a rapid delivery pipette. The air is gradually turned on and after a slow stream has passed through the tubes for five minutes the second acid tube in each series is connected with a water manometer which measures the rate of air flow. The valves are turned until a rate of five and a half liters per minute is attained. Thirty minutes aeration at five and a half liters per minute is required. The titrations are carried out directly in the acid tubes using approximately 0.01 N barium hydrate.

The difference between the titration value of the tubes containing urease and the tubes containing blood as well as urease gives the alkali equivalent of the ammonia produced from the decomposition of the urea in the blood.

One c.c. of 0.01 N barium hydrate is equivalent to 0.3 mg. of urea, so the number of c.c. of 0.01 N barium hydrate multiplied by 0.3 and by 50 will give the number of mg. of urea in 100 c.c. of blood.

In some laboratories there may be difficulty in securing a sufficiently constant air pressure. We had no compressed air supply available during the first few years, and after finding that water suction, no matter what apparatus was used, was inadequate, we filled a large tank with compressed air by means of a pump. But the needle valves which controlled the flow of air into the tubes had to be regulated at frequent intervals in order to maintain an approximately constant rate of flow. Even when a compressed air system was introduced, the varying use in other parts of the building induced too much fluctuation in the pressure in the laboratory. An entirely satisfactory and constant supply was only obtained when a pressure of 40 lbs. to the square inch was maintained by an automatically acting pump in a very large tank. This pressure is relayed to a large hot water tank in the laboratory and from it to two batteries each with eight needle valves. Even when all the sixteen outflows are being used the pressure remains constant and free from any measurable fluctuation.

APPARATUS

1. *Glassware.*—In this laboratory, urea determinations in six to twelve short time urine collections often have to be made, and we have found it best to have the subjects void urine in the laboratory directly into rubber stoppered bottles of about 1000 c.c. capacity. The name and the time over which the specimen was collected is written on the bottle with a grease pencil and it is placed in chronological series with the others on a table on which rows of Erlenmeyer flasks containing the buffer solution have been arranged three deep. If these positions are maintained it is not necessary to number the flasks. Very small amounts of mercury or of any heavy metal depress the activity of urease, so the flasks in the first row, into which the inactivated urease is pipetted, are permanently marked with black paint, and the flasks in which urea is decomposed are never used for any other titration. It is convenient to have these bottles and flasks set on a table which runs on rubber wheels so that when the urine volumes are measured or diluted or the flasks emptied and washed the table may be pushed up to the sink. When the work is done and the flasks have been rinsed with tap water they are set in the inverted position in holes bored in the top of the table.

The tubes used in the blood method are made from pyrex tubing and are sixteen and one-fourth inches long and have an internal diameter of one and one-eighth inches. They are closed with a No. 6 rubber stopper, through which pass two tubes of 4 mm. internal diameter, one running to the bottom of the tube and the other a short bent tube for the exit of the air during aeration. With these long tubes and with the volumes of fluid we deal with, there is no need of any device to break up the bubbles of air, nor is it necessary to employ any mechanism to prevent the carrying over of fluid from one tube to another.

The tubes are carried in wooden frames each of which holds four sets
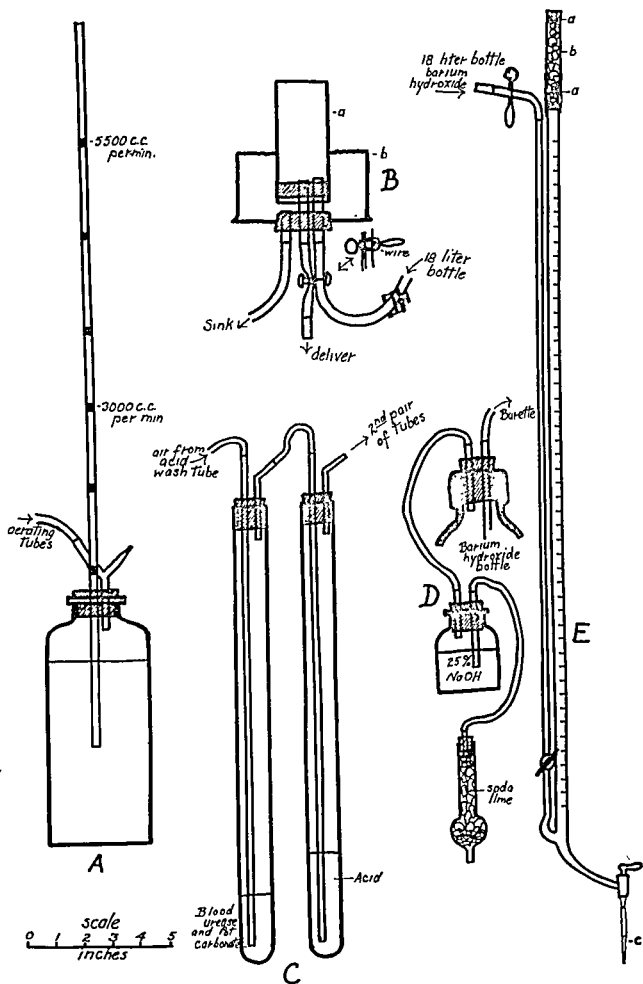
Fig. 1.—*A*, Meter for determining rate of air flow during aeration. *B*, Automatic device for rapidly delivering 100 c.c. of buffer solution: *a*, large glass tube; *b*, bottom of a large tin can. *C*, tubes set up for aeration, preceded by a tube of acid for washing the air (not drawn) and followed by tubes for blood and for acid. *D*, Means of protecting the barium hydroxide from carbon dioxide. *E*, Self-filling burette used for barium hydroxide; *a*, glass wool; *b*, soda lime; *c*, flexible tip. A similar burette without the carbon dioxide protection is used for the acid in the urine urea determination.

of three. They are conveniently set on a low wooden table which runs on wheels. The blood tubes are thoroughly rinsed with tap water after they have been used, and when not in use are always kept full of water. The neutral solution obtained at the completion of the titration with barium hydrate is left standing in the acid tubes until they are used again, when the fluid is run out and the tube is rinsed with boiled distilled water. When the tubes are new a little alkali may come from the glass, but in much-used tubes the yellowing of the neutral fluid which is left in them is due to the fading of the indicator.

2. *Self-filling Burettes.*—A great deal of time is saved by a device such as is shown in Fig. 1, whereby burettes can be rapidly filled. It is essential for the barium hydrate solution which, of course, would rapidly grow weaker if exposed to air containing carbon dioxide. With an adequate protection by soda lime tubes the strength may remain unaltered for weeks, though it should be checked at intervals by titration against acid potassium phthalate.

3. *Automatic Measuring Device for the Buffer Solution.*—This is very easily made and saves time in filling the flasks. In Fig. 1 the mechanism is drawn to scale.

4. *Measurment of Rate of Flow of Air.*—This is simply a bottle of about 1000 c.c. capacity half full of water with a long tube running to the bottom through a rubber cork and a narrow tube for the egress of air. The height to which the water rises in the tube is proportional to the rate of air flow. The tube is calibrated by noting with a stop-watch the time required to displace water from a vessel of known volume inverted in water.

THE COLLECTION OF URINE AND OF BLOOD

In the collection of urine there is always a danger that the bladder will not be completely emptied even though the subject is asked to make the collection as complete as possible. The possibility that urine will be left in the bladder is considerable when the volume collected is less than 100 c.c., and diminishes as the volume increases until with 500 c.c. volumes there is likely to be very little error if the cooperation of the subject is obtained. When approximately hourly collections are being made, the production of a diuresis is, therefore, one of the necessary elements for the attainment of accuracy. In certain patients the volume of urine per hour will not exceed 100 c.c. even when large amounts of water have been taken, and under these circumstances it is better to collect urine at one and a half or at two hour intervals. The collections are made in a room which opens off the laboratory. A commode with a glass funnel emptying into a 1000 c.c. bottle is used for the women, while the men collect the urine directly in the bottles. The timing is made at the moment when the collection is completed, not when it is begun, and the subjects are usually asked to note this time and to deliver the collection immediately in the laboratory where their memorandum is checked. Hourly urine collections are made in series so that an error due to incomplete voiding of urine may often be suspected when a marked irregularity in the successive amounts of urea is found. The urine estimations are made within an hour or two of collection but if for any reason this is impossible a sufficient

amount of 5 per cent sulphuric acid should be added to make the specimen strongly acid. This is the only effective method we have found to prevent the bacterial decomposition of urea.

Blood is taken at the middle of the short time periods of urine collection, and if that is not done the successive blood urea concentration values are plotted on coordinate paper and the average blood urea concentration for each period is calculated from the mean of the ordinates.

There are few exceptions to the general rule that it is a waste of time to work with urine and blood which has been collected by nurses or internes. Their other duties and their general training unfit them for accuracy in timing.

REFERENCES

[1]Marshall: Jour. Biol. Chem., 1913, xiv, 283.
[2]Addis and Watanabe: Jour. Biol. Chem., 1916, xxvii, 249.
[3]Fearon: Biochem. Jour. Liverp., 1923, xvii, 84.
[4]Werner: The Chemistry of Urea, London, 1923.
[5]Dodge: Jour. Indust. and Engin. Chem., 1915, vii, 29.
 Dodge: Jour. Amer. Chem. Soc., 1920, xlii, 1655.
[6]Handbook of Chemistry and Physics: 1918, 293.

SOME OF THE MORE PRACTICAL METHODS OF EXAMINATION OF THE BLOOD IN TUBERCULOSIS*

By HENRY C. SWEANY, M.D., CHICAGO, ILL.

CONSIDERING the importance of the disease, there is too little information gained from a blood examination in tuberculosis. Owing to the fact that the blood offers a most important means for the study of diseases, it seems that a greater effort should be exerted in this field to assist the clinician in diagnosis, prognosis and treatment of our most universal and until recently, most prevalent disease. With this aim in mind, we have been doing extensive work in our laboratory on blood chemistry and to a limited extent along other allied lines. We wish, therefore, to bring to date the various reactions that have been serviceable in the past and add the results of more recent findings that we feel will be of use; discussing these topics for convenience under the headings immunologic, cytologic, and chemical.

No doubt the greatest possibilities exist in this field of immunology, although at the present time they have not become evident. There were many encouraging reports a decade ago concerning complement fixation in tuberculosis. Besredka, Craig, Miller, Inman, Bronfenbrenner and others reported flattering results. Petroff in his reports is a trifle more conservative. The work of Corper and Sweany and others at that time resulted in a more pessimistic outlook. After eight years I can say that my opinion has changed little. Over ten thousand tests have been performed in our institution, using

Corper's Antigen with more or less hit and miss results. Two thousand tests were tabulated and studied but there was noted no advantage over our previous reports so the work was not published.

At present, we are using the new Wassermann antigen which seems to possess many advantages over any other method. Wassermann works on the hypothesis that there are antibodies developed for both lipoids and proteins and therefore he combines a tetralin extract of the tubercle bacillus with nonspecific lecithin. More positive results are obtained with this antigen than we have obtained with any other antigen, although there exist certain discrepancies which will be reported on later from this laboratory. The fact is evident that by mixing an anticomplementary lipoid extract with an anticomplementary lecithin in certain proportions an antigen is obtained that possesses a "range" of greater dimension. This does not occur with either lipoid or protein extract alone. After a trial of seventeen different antigens, specific and nonspecific extracts of the tubercle bacillus and other bacilli none of them were found to possess antigenic value except where mixtures existed such as are present in Corper's or Besredka's antigen. The pure extracts possessed almost as much anticomplementary power as they did antigenic strength. Just why a "range" should occur on mixing we have no way of explaining unless it is as a result of binding complement. It may be that by more accurate and refined technic with more specific antigens we may yet attain the long-sought method for early diagnosis along these lines, but until then the complement-fixation reaction in tuberculosis must be evaluated with a great deal of caution. The positive tests may not always be specific and the negative tests may occur in almost any stage of the active disease—more especially in the later stages. Before the reaction can become reliable the anticomplementary substances must be reduced to insignificant traces, otherwise the reaction will be due in part to anticomplementary factors and the remainder a combination of specific or nonspecific factors produced by a complex of substances that are adsorbed to the complementary aggregate and prevent it from acting.

Precipitin and agglutinin reactions have never given results worthy of confidence. Our results with the precipitin test on two hundred cases were approximately of equal value to those obtained with the complement-fixation reaction although by no means did they run parallel. The precipitin reaction was performed by making a ring test in a small tube by superimposing Corper's antigen over the serum and reading the reaction after two hours' residence of the tubes in a Uhlenhuth rack.

More recently the Wildbolz reaction gave promise of value but this test has also been shown to have many inherent faults so that it is doubtful whether these will be eventually overcome.

Thus we leave the field of humoral antibodies in tuberculosis with practically nothing dependable accomplished in so far as laboratory reactions of practical value are concerned.

Turning now to cytologic examinations, there are certain of these that merit consideration if properly performed and carefully interpreted. The Arneth nuclear classification indicates the trend of the disease but is by no

means constant enough to afford material aid. Likewise a red, white and differential count on the blood gives a limited amount of information. Only when performed and interpreted as we propose to show, is there sufficient information gained to warrant doing them. In Table I are listed the examinations in eight patients all having about the same quantity of massive involvement but varying in type of the process. The first patient is what may be classed as a healed fibroid and each one succeeding in order is of a more exudative state. The last patient died three days after the examinations were made. The Group V patients are fibroids that have healed or are healing; Group II are stationary and Group I are the exudative types or proliferative types that have become exudative. Of the patients that lived, most have been observed for several years

Beginning in the first column it is noticeable that the percentage of red cells gradually decreases from about normal, 45 per cent, to 30 per cent, a decrease of about 30 per cent. Presumably the converse is true if the patient recovers. The red cell count, however, changes only about 10 to 15 per cent. By combining these two values the volume index described by Capps[1] is obtained where the actual volume of each red cell is obtained expressed in cubic microns. This result is more significant. The volume of the red cell decreases from a normal of $90\mu^3$ to about $68\mu^3$ in the terminal stage. By multiplying these two results and dividing by 100 an exaggerated figure is obtained which may be termed the efficiency index. Those cases that fall below 30 are failing or not doing well. These results may be obtained by means of a red cell count and an hematocrite reading. The latter must be performed in a high speed instrument for eight to ten minutes at 6000 to 8000 r.p.m. or one hour at 2500 r.p.m.

The hemoglobin and color index are of some value but they are not as constant as the "efficiency index."

The white blood cells fluctuate in various ways and in general are not altogether satisfactory. The "lymphocytic index" is perhaps of most value and offers a fair gauge of the patient's condition. The total disappearance of eosinophiles is a sign of bad prognosis. The Arneth count has been mentioned before. The total white cell count is not consistent. The "transitionals" and the large mononuclears, however, are of definite significance and may be used to indicate the progress of the patient. If the large mononuclears are less than 1 per cent, with the transitionals less than 6 per cent, the condition is satisfactory or good; if the large mononuclears increase to 2 to 6 per cent the condition is less favorable; if in addition, the transitionals increase to 8 to 12 per cent the condition is unsatisfactory; and just before death both disappear from the blood. We can agree with Cunningham[2] and others in the origin of the transitional cell. In certain cases of advanced tuberculosis there is a good demonstration of this fact. It seems that when a patient suddenly experiences an acute exacerbation there is a sudden switch from the large mononuclears to the transitionals. During this time a complete series has been repeatedly observed from the large mononuclear through to the fully developed "transitional" or "endothelial leucocyte" of Mallory, showing that

TABLE I

CYTOLOGIC EXAMINATIONS OF THE BLOOD OF VARIOUS TYPES OF FAR ADVANCED TUBERCULOSIS

NAME	GROUP	% CELLS	R.B.C. PER M.M.	HB.	COLOR INDEX	VOL. INDEX	EFFICIEN-CY INDEX	W.B.C. PER M.	POLYS.	LYM.	EOS.	L. MON.	TRANS.	ARNETH
J.O'B.	V	45.6	4.85	94.0	1.00	91.0	41.5	6.8	71.0	25.0	2.0	0	2.0	45.6
T.H.	V	41.5	4.70	75.0	0.89	88.0	41.0	9.3	[72.0]	20.0	5.0	0	3.0	[60.0]*
R.M.	V	39.0	4.52	75.0	0.92	86.0	31.4	10.2	57.0	26.0	1.0	2	3.0	
J.G.	II	34.6	4.26	51.0	0.69	81.0	28.0	15.0	79.0	21.0	1.0	6.5	8.0	71.9
F.E.	II	35.0	5.77	85.0	0.82	64.0	22.4	12.1	73.0	11.5	2.0	3.3	9.4	71.0
J.Dad	I	36.8	5.04	69.0	0.77	73.0	24.8	11.4	61.0	22.9	1.0	2.0	11.3	75.0
J.Dog	I	28.0	4.23	37.0	0.49	66.0	18.5	22.4	79.0	7.7	0	4.8	10.5	74.0
A.O.	I	30.7	4.56	68.0	0.83	67.0	20.7	11.5	78.0	14.0	0	0	3.0	85.0

the stimulation has become an irritation and has caused an increased number of the irritation forms of the cell.

While there are valuable clues relative to the patient's condition obtainable from immunologic and cytologic data, it is my opinion that ultimately a chemical examination of the blood will furnish the most information. Quite recently we[3] carried on chemical studies on the blood of tuberculosis individuals with certain interesting findings, the most important of which was the "unbalance" of blood lipoids. In tuberculosis, as with certain other diseases, the toxemia appears to throw the various lipoids out of normal equilibrium. This unbalanced condition prevails during the various vicissitudes of the disease and does not come to normal until a complete recovery ensues. As this condition can only occur in mild tuberculosis it means that the lipoids remain out of normal balance during a great part of the time in far advanced tuberculosis. We found that cholesterol, and to a less extent lecithin and fats, fluctuated above and below normal, approximately in proportion to the proliferation or exudation present, and that a rising or falling lipoid value was a fair index of the patient's condition.

Continuing this work in a study of phosphorous compounds McCluskey[4] has shown that the lecithin and acid-soluble phosphorus play a reciprocal rôle. As the lecithin rises and falls the acid-soluble phosphorus fluctuates in the opposite direction. There is never any great change in the total phosphorus but the reciprocal relation of the two groups of ingredients furnishes valuable information relative to the patient's condition. She showed that the first changes took place in the plasma followed by a change in the cells. In other words the cells act as a storehouse from which the plasma draws when in need and stores up when there is an excess.

Still more recently Sweany[3] has studied the cholesterol and cholesterol ester partition between cells and plasma. Of these compounds the fluctuation between cells and plasma is very similar to that of phosphorus, but the esters show the greatest fluctuation and appear to be the most valuable index of any of the blood ingredients thus far studied. They certainly furnish the most valuable information from the clinical laboratory standpoint, because the greatest changes occur in the plasma (in fact, there are practically no esters found in the cells) and the changes are so marked that they are more readily interpreted. The total cholesterol in the whole blood varied from the low figure of 115 mg. per 100 c.c. of blood in the exudative types to over 300 mg. in the proliferative. The total cholesterol in the cellular portion of the blood ranged from 63.0 to 116 mg., while in the plasma portion the figures ranged from 51.0 to 253 mg. The cholesterol in the cells is almost all free, leaving the plasma to contain most of the esters. The free cholesterol in the plasma ranged from 29.0 mg. to 142 mg. but this constituent in plasma is inconstant. One arrested patient had practically no free cholesterol in the plasma. The esters, however, were more constant, ranging from 22.0 mg. to 176 mg. This latter ingredient in the plasma, therefore, appears to be of far greater significance than any of the others. If the values are calculated on 100 c.c. of plasma the results appear far more exaggerated, giving values that range

between 33 and 300 mg. The ratios between the extreme types of the disease are greatest when calculated on this basis.

Any information relative to the explanation of these changes is lacking. We merely know that they take place in tuberculosis. Luden[6] has reported lipoid changes in cancer. Perhaps every disease affects the balance of the lipoids to a more or less degree. But so far as tuberculosis is concerned, there are definite changes that take place concomitant with the condition of the patient. Whether the changes are a result of toxemia causing a change of cell permeability or colloid stability, a defensive mechanism, or simply the result of the presence of pus, we are still unable to answer.

We attempted to get some light on the latter mentioned point by analyzing sputum in different stages of the disease. We found that the cholesterol content is proportionate to the leucocytes, that it is approximately the same in the leucocytes as in the blood of the individual and that there were practically no esters present. It is conceivable that a patient loses the cholesterol (and lecithin which is present in about 1 per cent concentration) by expectorating a liter of sputum a day. This would be equal to about 1 gram of cholesterol and 10 grams of lecithin. On a low lipoid diet in advanced tuberculosis it is quite possible that the lipoids are thus depleted. The changes in the proliferative types are not so readily accounted for on this basis, because there is not a constant accumulation of pus to give the increase. It is true there is a gradual diminution of sputum and the system may be so adapted to the "forced draft" of lipoid metabolism that it goes on producing them at an increased rate until a gradual readjustment takes place. Neither do lipoids parallel a leucocytosis. In the patient that is not expectorating, yet where there is enough toxemia to produce an excess of leucocytes, there may be (but not necessarily) a parallel lipoid change. The diet cannot be responsible for it because the patients that gave such high results were on the same general diet as those that gave lower results. Although the source of lipoids is more exogenous rather than endogenous, yet the actual level is regulated by conditions within, possibly by the endocrines as suggested by Luden.

As the patient begins to approach normal the cholesterol is possibly changed to esters and oxidized or prepared for elimination; thus do we have the changes taking place that we have cited above.

SUMMARY

1. A review of the more practical laboratory procedures in blood examination in tuberculosis is given. There is nothing reliable in a diagnostic way, although definite prognostic aid may be obtained by certain carefully performed examinations.

2. In the immunologic field there is little to look forward to at present. Complement fixation must still be considered in the experimental stage. It only offers indefinite aid prognostically.

3. In the cytologic field the percentage of red blood cells, taken with the individual cell volume (volume index), the "lymphocytic index," the eosinophile count and an accurate study of the endothelial type of cells (large

mononuclears and transitionals) are all points that may be used in prognostication.

4. Of the blood chemistry reactions, the lecithin, acid-soluble phosphorus, cholesterol and cholesterol ester determinations on the plasma furnish the simplest and most practical blood chemical methods, and ones which we have found to be of definite prognostic aid.

BIBLIOGRAPHY

[1]Capps, J. A.: Jour. of Med. Res., x, 367.
[2]Cunningham, R. S.: Am. Rev. of TB, 1924, IX, No. 6, p. 491
[3]Sweany, H. C., Weathers, A T., and K Lucille McCluskey: Am. Rev. of TB., January, 1924, viii, No. 5
[4]McCluskey, K. Lucille: Jour. Lab. and Clin. Med., November, 1924, x, 143.
[5]Sweany, H. C.: Amer. Rev of TB. In Press
[6]Luden, Georgine M.: Abstract of thesis submitted to the Faculty of the Graduate School of the University of Minnesota, impartial fulfillment of the requirements for the degree of Doctor of Philosophy in Pathology, 1920

ODD LABORATORY APPLIANCES*

By Francis Lowell Burnett, M.D., Boston, Mass.

THE work of a clinical laboratory generally consists in the application of instruments of precision to the determination of the tissues, injurious agents, or forces of the body. In most instances articles on this subject deal with the results obtained from the use of instruments and appliances, and this of course is the most valuable part of scientific investigation. But the apparatus used may in some measure add to the accuracy and facility with which the results are obtained, and therefore I wish to illustrate and describe a few odd laboratory appliances with the hope that some of you may find them useful.

THE LABORATORY BENCH

(1) The laboratory bench contains a few new features. It extends along two sides of a room in a level and continuous line with the soapstone sinks and draining boards as shown in Fig. 1. The top of the bench is twenty-two inches wide and thirty-two inches above the linoleum. A powerful centrifugal machine is conveniently placed in the corner of the bench and the top projects a little above its surface. Along the front is a facing five inches wide and set back an inch from the outer edge of the bench. The front of several drawers forms part of this facing, but above them and immediately below the bench is a gas pipe (1). On this line of pipe there are numerous cocks (1) which control burners at the back of the bench. Then there are also electric switches (2) that operate outlets, the centrifugal machine and the daylite lamp. In a few convenient places there are rings

to which safety pins are attached (2). A large pin is used for holding a towel: and a small one by piercing the edge of several sizes of filter paper keeps them in an orderly and handy position. The gas outlets by means of an elbow have a horizontal attachment for rubber tubing or else are attached directly to a curved Bunsen burner (3) which pierces a soapstone gutter at the back of the bench.

THE GUTTER

This gutter is six inches across and four inches in depth. A galvanized 1-inch L iron is bolted to the back to support rods and asbestos mats for
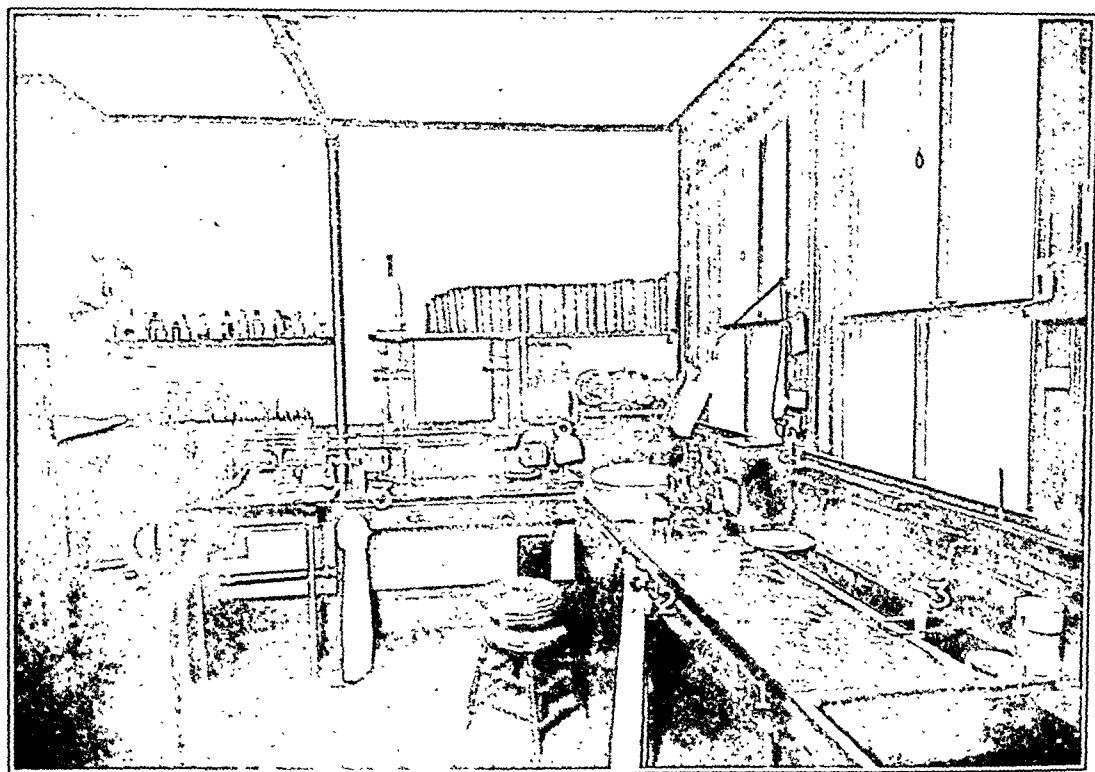
Fig. 1.—A general view of laboratory bench showing the gutter, the daylite lamp, the top of centrifugal machine (Corner), the gas and electric controls (2) on facing, and the gas outlets (3).

heating flasks and pans of fluid. The main purpose of the gutter, however, is to catch the drip from steaming stains and wash water from the small bibcocks above. The gutter has a drain that can be plugged. On a seat in front of part of the bench a worker can operate various burners, or electrical devices, and can stain, steam or wash smears without moving.

SOAKING BASIN

At each end of the bench there is a soapstone draining board and sink. The larger sink is divided by a partition, which encloses a soaking basin of 20 x 12 x 5 in. This has its own hot and cold water cocks, and also a plug for the drainpipe. This basin forms a convenient receptacle for soaking dirty and used glassware.

DRYING RACK

Immediately above each sink is a drying rack, Fig. 2, (4), which is made on the principle of a milk-can horse commonly seen outside the farm houses in New England. In this instance however it is made with a concavo-convex surface with short pegs, one, two and three inches long and one-fourth, three-eighths and one-half inches in diameter. It may be made of any length or width, although a projection of five inches from the wall is sufficient not to interfere with other appliances The pegs, too, may be placed at any distance from one another according to the glassware to be dried or stored. Such a device not only furnishes a convenient rack for
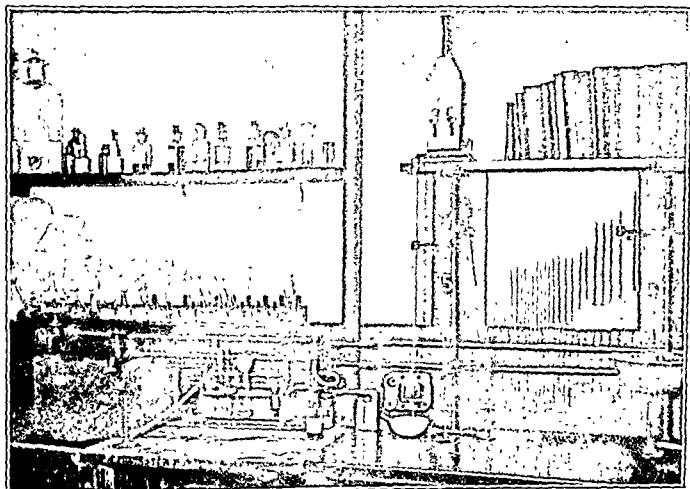
Fig. 2.—A close view of the bench showing the drying rack (4) the fixed ring and clamp uprights (5), and the volumetric pipette holder.

drying, but also a very compact and convenient means of keeping clean bottles, volumetric flasks and tubes. The volumetric pipettes are also very conveniently kept in order at the back of the water pipes.

CLAMP AND RING UPRIGHT

The metal support to which rings, clamps, etc., are attached, is a cumbersome piece of apparatus with a clumsy and heavy base that often takes up a great deal of room on a bench. To obviate the difficulties encountered with a movable stand, fixed uprights—Fig. 2, (5)—of one-eighth of an inch galvanized iron pipe are attached to the surface of the bench at one end and support a bookshelf at the other. Such a permanent and fixed upright is not only convenient and useful, but it furnishes an unusually steady sup-

port for rings holding pans of fluid, and also clamps for large tubes and burette.

DAYLITE LAMP

Since the invention of daylite glass several lamps have been put on the market for the illumination of laboratory apparatus. Most of them are small and adapted only for lighting a microscope. With a large lamp having an
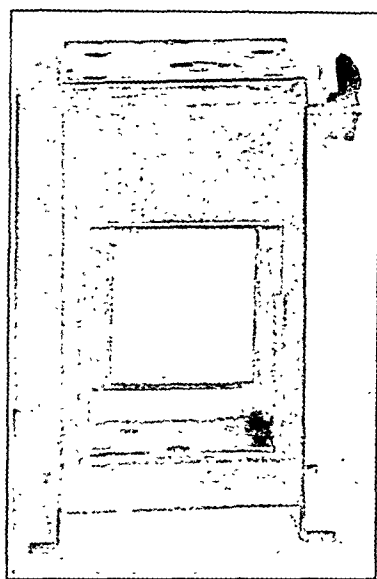
Fig 3.—A daylite glass lamp to use for a microscope, colorimeter or dark-field illumination.
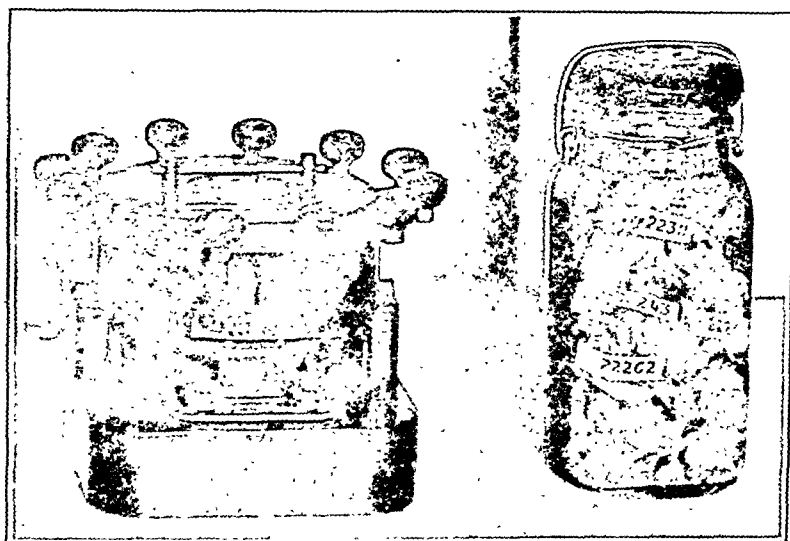
Fig. 4.—A perforating machine to number tags for tissue, and a jar containing some of the fixed, preserved and numbered specimens.

illuminating area of sixteen square inches as shown in Fig. 3, a constant and good light can be had at all times for the microscope and colorimeter. Then, too, the daylite glass window can be raised and the very intense light of a 200-watt nitrogen filled bulb used for a steady and good dark-field illumination. The body of the lamp case measures $10 \times 7 \times 6$ in. outside and is supported by four legs an inch high. In the permanent floor and the movable

top is a double layer of metal with three-fourths inch holes. The six holes in the layers of the top and bottom are not apposed, so that while the inside of the lamp is well ventilated the rays of light do not project outside. Across the interior there is a metal bar which supports the electric light socket from which wires run to a plug on the outside. The inside of the case is silvered and there is a reflector at the back of the lamp.

NUMBERING AND PRESERVING TISSUE

In many laboratories, especially those in which research and teaching are carried on, there are shelves and shelves of numbered or labelled bottles containing pieces of tissue. After the tissue is put into the bottle it is not often wanted again. On the other hand if the pieces of tissue are pierced with a thread and tied to a label of tough paper which is numbered by a check per-
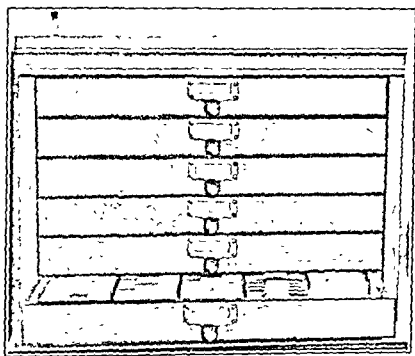
Fig. 5.—Cabinet for mounted and stained sections in which each drawer contains 750, and the case 4,500 slides.

forating machine (Fig. 4), all of the specimens can be put into a large receptacle. If the pieces of tissue are too small or friable to be strung on a thread, they may be put into a piece of gauze which is bound up with a thread piercing the label. In this way a great many bottles and corks and a large amount of alcohol will be saved. A quart jar filled with many numbered specimens is shown in Fig. 4.

A CABINET FOR MOUNTED SLIDES

Stained sections of tissue are also conveniently stored in a case of drawers illustrated in Fig. 5. This cabinet is of a standard make of the demi unit type,* and on this account innumerable sections may be obtained and placed one on top of another. Each drawer is divided by four partitions which form five rows in which the slides are nicely fitted. One hundred and fifty slides of medium thickness and some index cards can be kept in each row. With

*Library Bureau Legal Blank Demi Unit No 9726.

TRANSACTIONS

DR. J. H. BLACK, DALLAS, TEXAS, read a paper entitled **Pollen Sensitization: A Preliminary Report.** (For original article, see page 378.)

DR. O. J. WEST, SEATTLE, WASH., read a paper entitled **Is Pathologic Nomenclature Either Scientific or Sensible?** (For original article, see page 384.)

DR. FRANCIS LOWELL BURNETT, BOSTON, MASS., read a paper entitled **Odd Laboratory Appliances.** (For original article, see page 415.)

DR. HENRY C. SWEANY, CHICAGO, read a paper entitled **Some of the More Practical Methods of Examination of the Blood in Tuberculosis.** (For original article. see page 409.)

Doctor Corper.—I have not very much to add to Dr. Sweany's presentation, except that I should like to point out the significance of this contribution in tuberculosis, a point which I believe was not emphasized in the presentation.

This paper has at least one important significance aside from the practical points dwelt upon by Dr. Sweany. For the past fourteen years Dr. H. G. Wells and his colleagues have been interested in the chemistry of tuberculosis and as a culmination of their studies and a review of reports in the literature have contributed recently the first and a most excellent text volume on this phase of medicine. The information on blood chemistry had to be taken from many different sources and as such was patched information and not entirely satisfactory, many studies having been made with obsolete and inaccurate methods. Dr. Sweany and his colleagues have presented to us a complete study of the blood by the most modern and approved methods for blood chemistry. From this standpoint, you can readily appreciate that the work is not only of practical, but also of definite original scientific value.

I wish to compliment Dr. Sweany and his colleagues for this contribution to our knowledge in tuberculosis.

The Journal of
Laboratory and Clinical
Medicine

EDITORIALS

The Hospital Laboratory: Its Scope, Function and Relation to Hospital Efficiency

MODERN studies in hospital efficiency may be summed up in the somewhat obvious conclusion that hospital efficiency is fundamentally dependent upon the efficiency, not of any special departments, but upon the efficiency of the hospital as a whole.

The functions of the modern hospital, regardless of size, are multiple and indivisible and closely linked with the general efficiency of the hospital as a whole; all are of equal importance and none capable of undue accentuation at the expense of the others. They embody much more than the housing of the sick. They may be looked upon as: (1) The scientific, skilful, and ethical care of the sick; (2) Teaching, as concerns the nurse, the interne, the staff, and the community; and (3) Research, as concerned with the evaluation of old methods, and the evolution of new—whether concerned with the diagno-

sis or treatment or with the better understanding of the mechanism and manifestations of disease.

The function of the hospital laboratory in the practical realization of these objectives is, or should be, obvious. The scientific care of the sick; intelligent and effective teaching; the acquisition and dissemination of knowledge. Productive studies and constructive research do not and cannot result from the accumulation or analysis of isolated facts.

Diagnosis, the *sine qua non* of intelligent and scientific treatment, teaching and research, depend, not upon the accumulation of a mass of isolated and perhaps not obviously related facts, but essentially upon their interpretation and application to the particular problem; upon the correlation of one fact with another.

The scientific, careful, or even the intelligent study of the problems confronting the hospital in the fulfilment of its duties or functions is impossible without the active cooperation of the laboratory. That every case, without exception, entering the hospital should be subjected to certain fundamental laboratory studies is now generally admitted; many will demand further and more extensive investigations, but none can escape or fail to utilize the absolute minimum. The laboratory, however, which confines its work solely to the collection and issuance of a mass of statistical data fails entirely in its true function, for the laboratory and the wards, the laboratory and the hospital, cannot be separated successfully.

The laboratory, through its studies, may bring forth and establish certain facts as suggested by the presence or absence of certain findings, but the laboratory, through its director, should also have an active part in the correlation of these facts with the others also elicited in the particular case; unless it is in liaison with all the other departments engaged in the study of a particular problem, its full duty and its highest efficiency fail of accomplishment.

Proper, complete, and active liaison between the laboratory and the clinical departments of the hospital is essential as, also, is the recognition of the fact that, specialized as the practice of medicine has become, there is no specialty so distinct as to enable it to withdraw from contact with medicine as a whole; the proper understanding of one necessitates, at least, an intelligent conception of the others.

Without a properly functioning laboratory, a properly functioning hospital is impossible and the proper functioning of the laboratory depends, not upon the number of reports issued, but upon the degree to which it is concerned with, and utilized in the study of disease and the interpretation and analysis of its manifestations.

It is plain that this conception necessitates the entrance of the laboratory into the wards, as it were, and its active and consulting participation in clinical problems.

It has been stated by Stillman:[1] "What should be the attitude of the toward requests for examinations made by the clinical division? merely perform the examinations and say nothing * * * or trude itself into the wards, urge the adoption of some methods

and the dropping of others, and generally cooperate in the attempt to solve the clinician's problems? Obviously, the latter position is the only one tenable if the laboratory is to be of the greatest possible service in every respect.'' If the former only is accepted, then ''all that is necessary is the installation of sufficient technicians to do the work.''

The modern clinician realizes that the laboratory and its personnel constitute a highly specialized department of the hospital and that the hospital comprises a mechanism composed of highly specialized units working in coordinated harmony. The efficiency of the hospital laboratory is mainly influenced by and dependent upon the ability and efficiency of its director, who should be chosen, not merely for his manipulative efficiency in various technical laboratory procedures, but for his ability to supervise the work of his assistants, to eliminate lost motion, and, above all, for his perspective, vision, and ability to coordinate and correlate the work of his department with the work of the clinical divisions.

The director must, therefore, have had a well-grounded training in the clinical arts in order that he may be able to interpret in terms of the patient the pathology or abnormalities he demonstrates in the laboratory—which, in essence, constitutes the difference between the clinical pathologist and the technician, however competent or expert the latter in technical manipulations.

The College of Surgeons has recognized these necessities in including among the minimum hospital requirements that a clinical laboratory in charge of a clinical pathologist must be ''available''—but there is still, in places, a hiatus between the availability and the utilization of these requirements.

Every hospital will have its own problems, each laboratory its particular difficulties; the solution of the problem lies in a recognition of the fact that laboratory and clinical medicine are indissolubly linked together; that the laboratory is—or should be—a highly specialized department of clinical medicine; that ''the pathologist is—or should be—a well-trained clinician and diagnostician equally well trained, in addition, in the performance and interpretation of laboratory investigations * * * a consultant from the standpoint of *what* to do as well as how to do it,''[2] and that it is the duty of the clinician to be sufficiently acquainted with the advances in ways and means of laboratory investigations to use them to the best advantage.

REFERENCES

[1]Stillman, R. G.: A Criticism of Hospital Laboratory Examinations, Jour. Amer. Med. Assn., June 25, 1921, lxxvi, 1816.
[2]Kilduffe, R. A.: Function of the Pathologist as a Consultant, Jour. Amer. Med. Assn., January 1, 1921, lxxvi, 54.

—*R. A. K.*

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The Next Annual Meeting Will Be Held in Philadelphia May 21-23, 1925
Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 21, 22 and 23, 1925, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering next year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.

CLINICAL AND EXPERIMENTAL

MYOCARDIAL INFARCTION: AN ELECTROCARDIOGRAPHIC STUDY*

A Report of Nine Cases from the Mayo Clinic, and a Review of Twenty-four Published Cases

By Frederick A. Willius, M.D., and Arlie R Barnes, M.D , Rochester, Minn.

THE clinical syndrome accompanying sudden obstruction of the coronary circulation is not always typical, and hence not infrequently leads to diagnostic error. It is important, therefore, that all cases of this type be carefully studied with special reference to the correlation of symptoms, physical findings, and special lines of cardiac investigation, such as electrocardiography and necropsy. The scope of this paper does not permit a detailed discussion of the clinical features of myocardial infarction which have been repeatedly and comprehensively recorded in the literature.[1, 2, 3, 10] But in view of the fact that few representative series of cases with electrocardiographic study have been recorded, we have incorporated our group of nine cases with those already published and base our conclusions on the aggregate group.

REPORTS OF CASES (WILLIUS AND BARNES)

Case 1 (A148902).—A man, aged fifty-seven years, was admitted to the hospital August 15, 1919, suffering with severe epigastric pain He had had typical attacks of angina pectoris for the last five years, which had increased in frequency and severity during the year preceding this admission. Four years before, the blood pressure was elevated, the systolic pressure being 162 and the diastolic 128. After his noon meal the day before examination, the patient was suddenly taken with severe, localized, persistent pain in the epigastrium. He vomited once, soon after the onset of the pain. His home physician had given six subcutaneous injections of morphine, varying from ¼ to ¾ grain, without relief.

Examination revealed an extremely ill man, who presented the facies of extreme suffering. The area of cardiac dullness was increased, extending 12 cm. to the left of

the median sternal line. The heart tones were very distant and heard with great difficulty, owing to noisy breathing. The abdomen was moderately distended and tympanitic, and tender in the epigastrium. The blood pressure was not obtained. The temperature was 102° F., and the leucocyte count was 34,200. The electrocardiogram disclosed T wave negativity in all derivations, with preponderance of the left ventricle (Fig. 1). The patient died one hour after admission to the hospital.

At necropsy the heart was markedly enlarged, the apex consisting largely of the left ventricle. The coronaries were prominent, tortuous and beaded. The aortic valve, especially around the attachments of the leaflets, contained a moderate amount of fatty and fibrous material with some calcareous deposits. The myocardium of the left ventricle was enormously thickened, in some places measuring 2.5 to 3 cm. The muscle was mottled chocolate, yellow and grey. In the upper half of the interventricular septum was a dark reddish area about 2 cm. in diameter, and softer than the rest of the myocardium. A similar area was found on the external surface of the heart on the
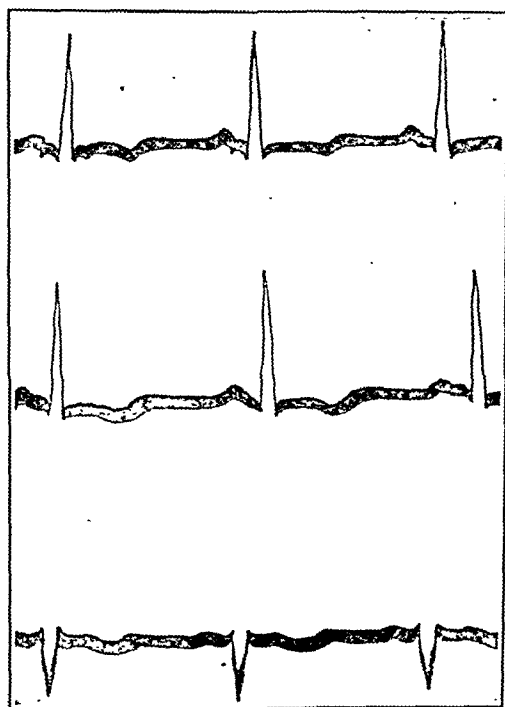
Fig. 1.—Case 1 (A148902). Taken about one-half hour before death. T wave negativity in Derivations I, II and III. Left ventricular preponderance.

posterior surface of the left ventricle, almost at the auriculo-ventricular juncture. A mottled clot was present in the apex of the left ventricle, and firmly attached to the papillary muscles. The coronaries were opened with great difficulty and the walls were found to be greatly thickened and to contain many nodular, yellow and grey plaques. The lumen in some places was practically occluded. Besides the myocardial infarction, there were multiple infarcts in the right kidney, and an embolism in the superior mesenteric artery.

CASE 2 (A298068).—A man, aged forty-two years, was admitted to the Clinic November 26, 1919, complaining of attacks of severe retrosternal pain following exertion. He had contracted syphilis in 1896 and received little or no treatment until two years ago. During the last seven years he had had severe attacks of retrosternal pain following exertion, or the ingestion of a rather heavy meal. The attacks had become more frequent and severe during the last three weeks.

Examination revealed a well nourished man, not particularly ill. His heart was moderately enlarged, and to-and-fro murmurs were audible at all areas, maximal at the

aortic area. Capillary and water-hammer pulse, and pistol-shots in the femoral arteries were noted. The liver edge was palpable 2 cm. below the right costal margin. The systolic blood pressure varied from 128 to 136, and the diastolic was 60. The urinalyses were negative. The hemoglobin was 74 per cent, the erythrocytes numbered 4,600,000, and the leucocytes 9,200. The blood Wassermann reaction was negative. Roentgenograms of the chest revealed moderate enlargement of the heart, particularly to the left. The first electrocardiogram taken three days after admission to the hospital showed a rate of 130, sinus tachycardia, negative T wave in *Derivation I*, and preponderance of the left ventricle. The T wave in Derivations II and III was markedly exaggerated (Fig. 2). A diagnosis was made of syphilitic aortitis with aortic regurgitation, myocardial degeneration with dilatation and hypertrophy, and angina pectoris.

The patient became progressively worse, and died of a progressive cardiac failure nine days after admission to the hospital. There was no fever, but the leucocyte count
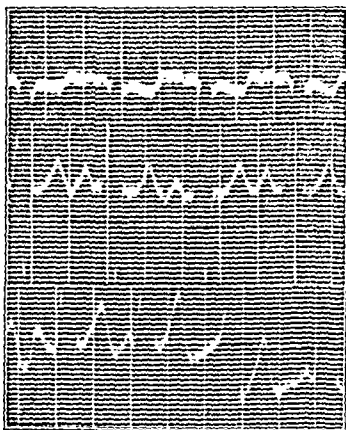
Fig. 2.—Case 2 (A298068). Taken three days after admission. T wave negativity in Derivation I. Left ventricular preponderance.
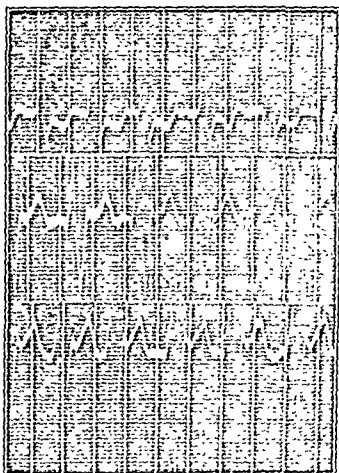
Fig. 3.—Case 2 (A298068). Taken four days after tracing in Fig. 2, and on the day preceding death. Rate 166. Nodal tachycardia. *T wave negativity in Derivation I.* Left ventricular preponderance.

the day before death was 16,600. The electrocardiogram taken on the same day showed a rate of 166, nodal tachycardia, occasional *ventricular premature contractions*, T wave negativity in Derivation I, and preponderance of the left ventricle (Fig. 3).

At necropsy advanced syphilitic aortitis was present. The anterior aortic cusp was scarred and somewhat adherent. The aorta from without was studded with oval projections, which were evidently early aneurysmal pocketings. In the apex of the left ventricle in the visceral pericardium was a hemorrhagic infarct about 2 cm. in diameter, which was much softer than the contiguous structures, and extended to the depth of 2 cm. The coronary arteries were patent and showed no sclerosis.

CASE 3 (A399583).—A man, aged sixty-five years, came to the Mayo Clinic July 28, 1922, complaining of attacks of retrosternal pain with radiation into the left arm on exertion. His only previous illnesses had been typhoid fever at the age of twenty-six,

and pneumonia six years before his examination. The attacks of retrosternal pain were becoming more frequent and severe, and at times he had severe attacks of dyspnea in which he feared he would die.

Examination revealed a well-developed man who was able to be up and about with relative comfort. The cardiac dullness was increased, extending 3 cm. to the right and 15 cm. to the left of the median sternal line. The rhythm was regular, but to-and-fro murmurs were audible at all areas; both were maximal at the aortic area. A capillary and water-hammer pulse was present, and pistol-shots were audible in the femoral arteries. The lungs were emphysematous. The prostate was slightly enlarged, firm and smooth. The systolic blood pressure was 192; it was impossible to determine the diastolic reading accurately. A detailed neurologic examination disclosed the findings of syphilis of the central nervous system.

The blood Wassermann reaction was strongly positive. The urinalyses revealed a small amount of albumin, an occasional hyaline and granular cast and a few erythrocytes and leucocytes. The hemoglobin was 85 per cent, the erythrocytes numbered 4,500,000
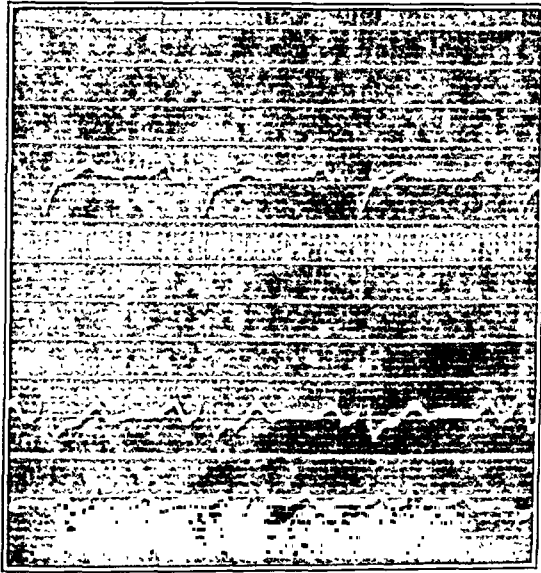
Fig. 4.—Case 3 (A399583). Taken three days after admission and twenty-three days before death. Sinus rhythm. Left ventricular preponderance.

and the leucocytes 6,200. The combined phenolsulphonephthalein test gave an excretion of 80 per cent of the dye in two hours. There were 34 mg. of urea and 15.8 mg. of urea nitrogen in each cubic centimeter of blood. Examination of the eyes revealed the Argyll-Robertson type of pupil and the fundi showed mild arteriovenous compression. Roentgenograms of the chest showed only the cardiac enlargement. An electrocardiogram taken three days after admission to the hospital, and twenty-three days before death, revealed only preponderance of the left ventricle (Fig. 4). A diagnosis of syphilitic aortitis and aortic regurgitation was made. The patient grew progressively worse and died of progressive cardiac failure August 23, 1922.

Necropsy revealed that the enlarged heart weighed 500 gm. The myocardium was flabby and on cut section had a mottled appearance. Over the right side of the anterior surface of the epicardium was a white opaque patch measuring 3 cm. by 2 cm. (soldier's patch). There was a diffuse thickening of the endocardium of the left ventricle, and over this were scattered small areas of erosion and ulceration and one large area measuring 3 by 1 cm. to which was attached a thrombus. This area had the appearance of an infarction. The aortic valve showed diffuse thickening and shortening of its cusps. The coronaries were markedly thickened with distinct encroachment of the lumen. The

aorta showed advanced arteriosclerotic changes, especially at the root with atheromatous ulcers, and mural thrombosis.

CASE 4 (A401659).—A man, aged seventy years, came to the Mayo Clinic August 16, 1922. His previous illnesses had been unimportant, other than malaria when a young man and influenza in 1918. He had been pronounced perfectly well three months before examination, and readily passed two life insurance examinations. Two weeks before, while undressing, he had a sudden, severe, agonizing pain behind the sternum and upper abdomen; his suffering was so extreme that he thought he was going to die. This pain lasted three days.

The patient had another attack of pain, identical to the first, the day after his arrival at the Clinic. He was admitted to the hospital at once. His temperature was
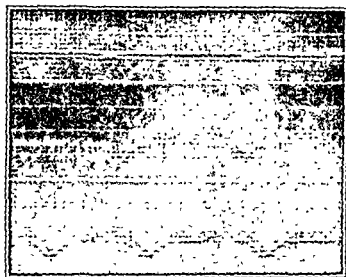
Fig. 5.—Case 4 (A401659). Taken during attack. T wave negativity in Derivations I, II and III.
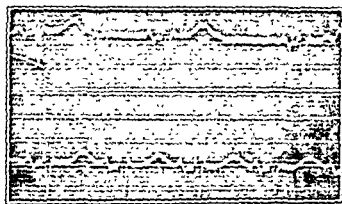
Fig. 6.—Case 4 (A401659). Taken same day as tracing in Fig. 5. Sino-auricular block. Both records in Derivation III.

101° F. His heart was greatly enlarged on the left, but the rhythm was regular and there were no murmurs. Morphine was given with only slight relief. An electrocardiogram taken at this time showed the T wave to be negative in all derivations, and periods of sino-auricular block and preponderance of the left ventricle (Figs. 5 and 6). The heart was definitely enlarged to the left, and an arrhythmia was present. The next day a distinct pericardial rub was audible over the lower sternum. The temperature receded to 99.8° on the second and third days, and then remained normal. The urinalysis was negative and the combined phenolsulphonephthalein test showed an excretion of 50 per cent of the dye in two hours. The blood Wassermann reaction was negative. The roentgenogram confirmed the cardiac enlargement. The blood count was normal. The systolic blood pressure was 108, and the diastolic 78. A diagnosis of acute coronary occlusion was made.

The patient recovered uneventfully and returned home at the end of two weeks. He returned for examination about seven months later (March 13, 1923) complaining of considerable shortness of breath on effort, and attacks of paroxysmal dyspnea at night. There had been no recurrence of anginal attacks.

Examination revealed the heart to be smaller than on the previous examination, the cardiac dullness extending 3.5 cm. to the right and 11 cm. to the left of the median sternal line. There was no arrhythmia and no murmurs. The systolic blood pressure was 110 and the diastolic 70. The electrocardiogram at this time showed the T wave to be negative in Derivation I, and left ventricular preponderance (Fig. 7).

CASE 5 (A42825).—A man, aged fifty-three years, came to the Mayo Clinic August 30, 1922. He had felt well up to July 1 except for mild indigestion to which he had been subject for ten years. On this day a sudden severe pain developed in the epigastrium, extending over the whole abdomen; the exact duration was not noted. Such attacks recurred at irregular intervals; the dyspnea developed, and was becoming progressively worse at the time of his admission.

On examination the patient appeared to be extremely ill. He was dyspneic, moderately cyanosed, and the dependent portions of the body were markedly edematous. The heart was enlarged, the dullness extending 4.5 cm. to the right, and 14.5 cm. to the.
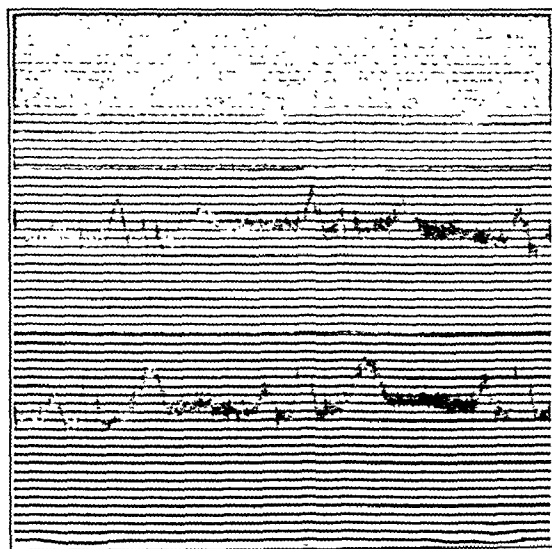

Fig. 7.—Case 4 (A401659). Taken seven months after tracing in Fig. 6. T wave negativity in Derivation I. Left ventricular preponderance.

left of the median-sternal line. There was total arrhythmia. The systolic blood pressure was 120 and the diastolic 100. A twenty-four hour specimen of urine contained 750 c.c., specific gravity 1.018, a faint trace of albumin, no sugar and an occasional leucocyte. The combined phenolsulphonephthalein test showed an excretion of only 15 per cent of the dye in two hours. The urea varied from 82 to 46 mg. in each 100 c.c. of blood. The roentgenogram of the chest confirmed the finding of cardiac enlargement, and showed a thickening of the pleura at both bases. The initial electrocardiogram taken on the third day after the patient's admission to the hospital (September 2, 1922) disclosed auricular flutter, the ventricular rate varying from 83 to 169, and the auricular rate from 327 to 338 (Fig. 8). Three days later the electrocardiogram revealed nodal tachycardia (Fig. 9). Two days later premature contractions were present and there was a tendency for fusion of the R and T waves in Derivations I and II (Fig. 10). The next day (the day of the patient's death), a delay in A-V conduction was noted, the P-R interval being 0.24 second (Fig. 11). This was probably a digitalis effect, as the patient had received 23 c.c. of the tincture in five days.

The patient was placed on the usual cardiac regimen, including the administration of the tincture of digitalis, but became progressively worse and died on the ninth day after admission to the hospital (September 7, 1922).

At necropsy the heart was markedly enlarged, weighing 925 gm. A large globular mass was visible at the apex, which measured 12 cm. in its greatest diameter. The pericardium was adherent to the anterior surface of this mass by dense fibrous adhesions,
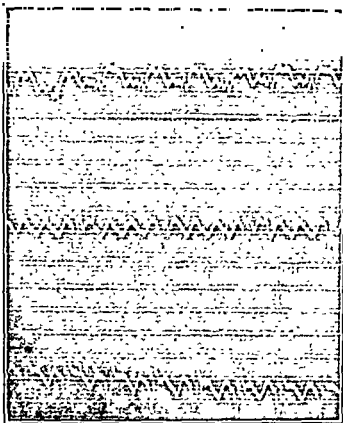

Fig. 8.—Case 5 (A42825). Taken three days after admission. Auricular flutter, ventricular rate 83 to 169, auricular rate 327 to 338 (2:1 to 4:1 block).
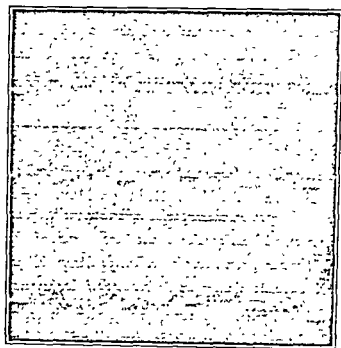
Fig. 9.—Case 5 (A42825). Taken three days after tracing in Fig. 8. Nodal tachycardia.
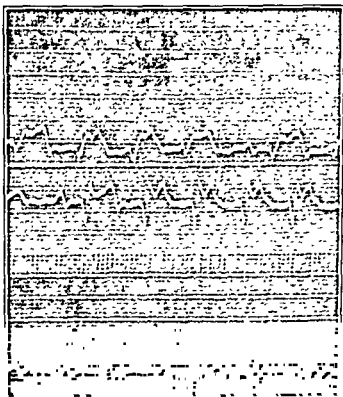

Fig. 10.—Case 5 (A42825). Taken two days after tracing in Fig. 9. Premature contractions. Tendency for fusion of R and T waves in Derivations I and II.
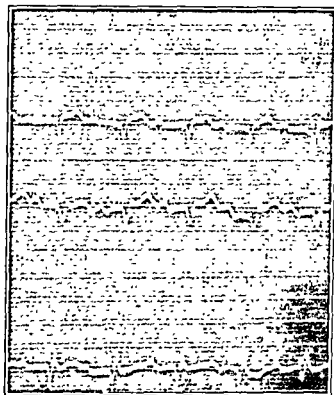

Fig. 11.—Case 5 (A42825). Taken day after tracing in Fig. 10. Delayed A-V conduction (P-R, 0.24 second).

and the wall of the mass consisted of a thin membrane. The anterior portion of the apex and the apex itself of the left ventricle were involved in this outpouching, and out of this pouch a round, flattened thrombus about 10 by 10 cm. came away. The margin of the pouch was sharply demarcated to the left ventricle, but a portion of the interventricular septum adjacent to it was whitish grey and firm. The descending branch of the left coronary artery was completely occluded by an old thrombus, just at the upper margin of the outpouching described.
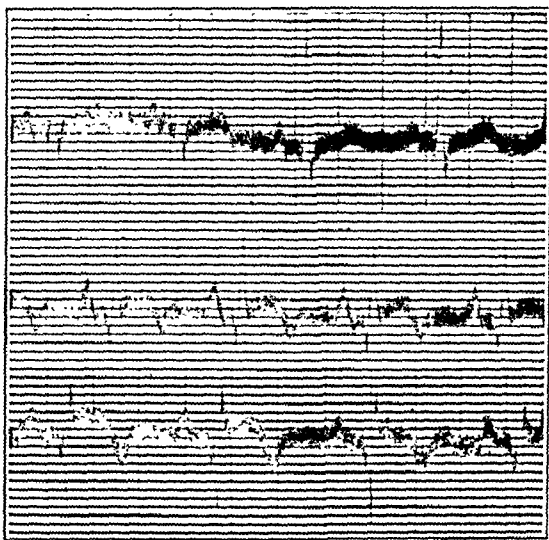

Fig. 12.—Case 6 (A425425). Taken two days after admission and before attack. T wave negativity in Derivations II and III.
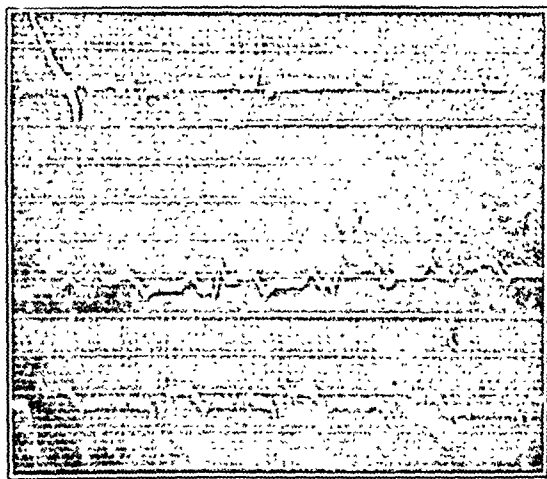

Fig. 13.—Case 6 (A425425). Taken during attack and four days after tracing in Fig. 12. Tendency for fusion of R and T waves in Derivations II and III. T wave negativity in Derivations II and III.
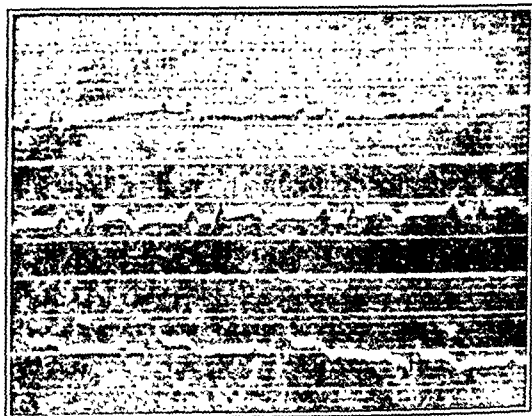

Fig. 14.—Case 6 (A425425). Taken day after tracing in Fig. 13. T wave negativity in Derivations I and II, and diphasic in Derivation III. Fusion of R and T waves has disappeared.

CASE 6 (A425425).—A man, aged forty-three years, was admitted to the hospital May 10, 1923, suffering from severe attacks of retrosternal pain. He said that his general health had been excellent until the onset of the present trouble in March, 1922, when suddenly, while exerting himself, he had a severe retrosternal pain which radiated over the chest, through to the back, and down both arms. This was relieved in a few moments by complete rest. Many similar attacks occurred afterward, following effort, and increased in severity.

Examination revealed the heart to be slightly enlarged, the cardiac dullness extending 3 cm. to the right and 11 cm. to the left of the median sternal line. There was no arrhythmia, and no murmurs. The systolic blood pressure was 116, and the diastolic 84. There was a moderate degree of peripheral arteriosclerosis. The urinalysis revealed only a faint trace of albumin. The blood Wassermann reaction was negative, and there were 32 mg. of urea in each 100 c.c. of blood; the x-ray confirmed the slight cardiac enlargement.

The electrocardiogram taken two days after admission revealed T wave negativity in Derivations II and III (Fig. 12). A very severe anginal attack occurred which lasted for thirty hours, and during this time the leucocyte count varied from 20,800 to 15,200, and the temperature from normal to 101.2°. The electrocardiogram taken during the attack showed the T wave to be negative in Derivations II and III, but in these derivations there was a tendency for the fusion of the R and the T waves (Fig. 13), and a diagnosis of coronary thrombosis was made. After this severe attack, a distinct to-and-fro pericardial rub was audible to the left of the lower sternum. The electro-
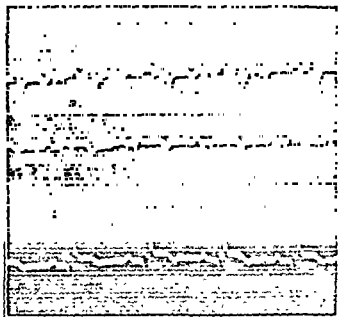

Fig 15.—Case 6 (A425425). Taken day after tracing in Fig. 14. T wave negativity in Derivations I, II and III.
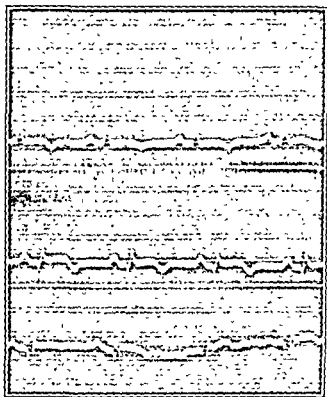
Fig 16.—Case 6 (A125425). Taken day after tracing in Fig. 15. T wave negativity in Derivations I, II and III.

cardiogram taken the next day showed the T wave to be negative in Derivations I and II, and diphasic in Derivation III; the fusion of the R and the T waves had disappeared (Fig. 14). On the second day, the T wave was negative in all derivations, (Fig. 15) and on the third, the same electrocardiographic findings were present (Fig. 16).

The patient recovered and was alive about six months ago, when he was last heard from.

CASE 7 (A448624).—A man, aged fifty-seven years, was admitted to the hospital November 26, 1923. He had been well except for an attack of mild influenza during the epidemic of 1917. For a year and a half before coming to the Clinic he had complained of shortness of breath on effort. This was moderate until two months prior to his admission, when under relatively little effort he became very dyspneic, particularly when he exerted himself soon after eating. In the early part of November, while driving his automobile, he suddenly developed a severe oppression in his chest, associated with marked difficulty in breathing, and on returning home he went to bed, and remained there for three days. On the day of his arrival at the Clinic, he had an attack

of severe retrosternal pain, associated with marked shortness of breath, which lasted for ten hours.

Examination revealed an enlarged heart, the cardiac dullness extending 3.5 cm. to the right and 11 cm. to the left of the median sternal line, rapid and distant heart tones, and a moderate degree of peripheral arteriosclerosis. The systolic blood pressure was 130, and the diastolic 90. The urinalysis was negative. The hemoglobin was 75
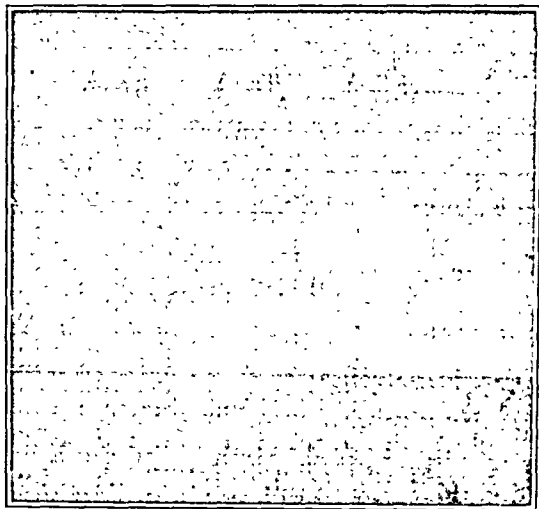

Fig. 17.—Case 7 (A448624). Taken during attack. Ventricular premature contractions. Notched QRS complexes in Derivations I and II, 0.12 second. T wave negativity in Derivations II and III.
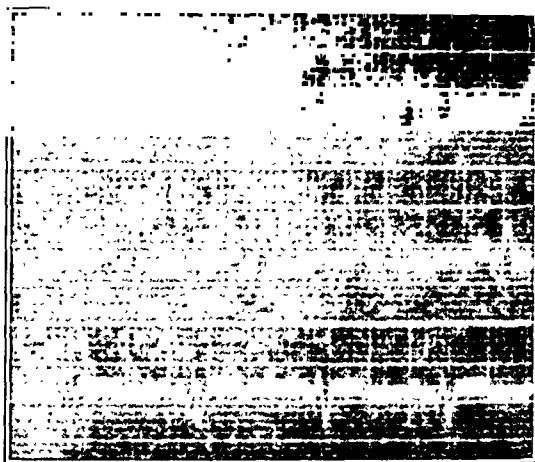

Fig. 18.—Case 7 (A448624). Taken day after tracing in Fig. 17. Notched QRS complexes in Derivations I and II, 0.12 second. T wave negativity in Derivations I, II and III.
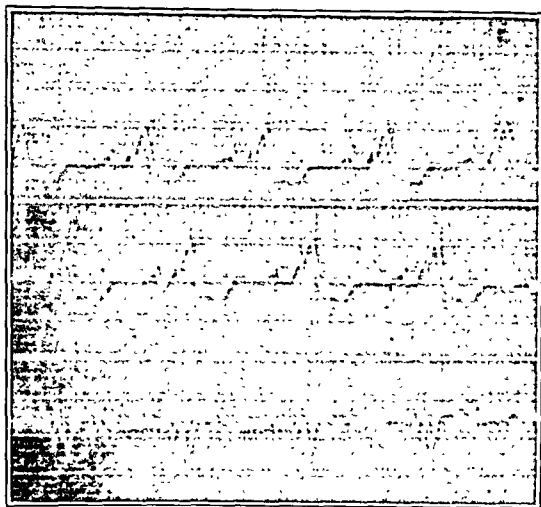
Fig. 19.—Case 7 (A448624). Taken two days after tracing in Fig. 18 and two days before death. Notched QRS complexes in all derivations, 0.12 second. T wave negativity in Derivations I and II.
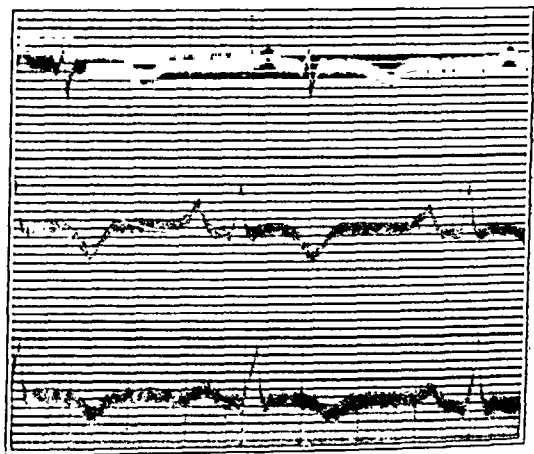
Fig. 20.—Case 8 (A130266). Taken forty-six days after attack. T wave negativity in Derivations I, II and III.

per cent; the erythrocytes numbered 4,840,000, and the leucocytes 7,500. An electrocardiogram taken during this attack revealed ventricular premature contractions, notching of the QRS complexes in Derivations I and II, the base width being 0.12 second (Fig. 17). There was T wave negativity in Derivations II and III. A diagnosis of acute coronary occlusion was made. The patient felt fairly comfortable the next day, and the electrocardiogram was similar to the one taken during the attack, except that the T wave

was negative in all derivations (Fig. 18). The electrocardiogram taken two days later showed a notching of the QRS complexes in all derivations, the base width being 0.12 second with T wave negative in Derivations I and II (Fig. 19). Two days later the patient suddenly developed another severe anginal attack and died in a few moments.

At necropsy the heart was found to be markedly dilated, and weighed 520 gm. The myocardium was firm and light red, and in the anterior surface of the left ventricle a sharply defined area of bright red, 2.5 cm. in diameter was found, which was distinctly
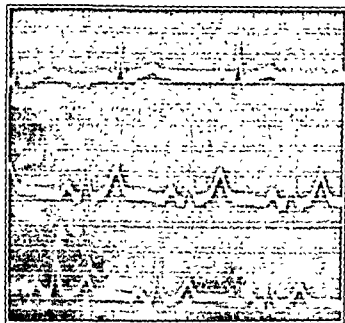
Fig. 21.—Case 9 (A461768). Taken during attack. Sinus arrhythmia.

Fig. 22.—Case 9 (A461768). Taken day after tracing in Fig. 21. Nodal premature contractions.
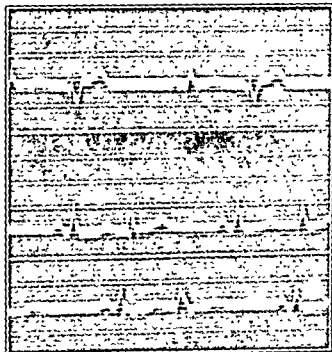
Fig. 23.—Case 9 (A461768). Taken day after tracing in Fig. 22. T wave negativity in Derivation I. Right bundle branch block.

a recent infarction. On the posterior portion of the left ventricular septum was found a scarred and thinned-out area about 1.5 cm. in diameter, the evidence of a previous infarction. There was marked sclerosis of the coronary arteries and a moderate degree of sclerosis at the base of the aorta.

CASE 8 (A130266).—A woman, aged sixty years, came to the Mayo Clinic December 6, 1923. The past history was unimportant; however, she had had a cholecystectomy in

September, 1917, for acute empyema of the gall bladder with cholelithiasis, from which she made an excellent recovery. Forty-six days prior to her examination at the Clinic, she had a sudden severe attack of retrosternal pain radiating into both arms, associated with nausea and vomiting, and intense pain for about eighteen hours. The next day her local physician discovered the presence of a pericardial rub, and the condition was interpreted as an acute pericarditis. She was kept in bed for a complete rest for three weeks. The week prior to her visit to Rochester she had two rather severe attacks of shorter duration, but at the time she was under our observation she was entirely free from pain, although under moderate effort, dyspnea was marked.

The heart was slightly enlarged, the cardiac dullness extending 4.5 cm. to the right and 12 cm. to the left of the median sternal line. There were no murmurs, but the tones were rather distant. The urinalysis was negative; the hemoglobin was 74 per cent, erythrocytes numbered 4,730,000, and the leucocytes 9,000. Roentgenograms of the chest confirmed the finding of cardiac enlargement. The electrocardiogram revealed T wave negativity in all derivations (Fig. 20). A diagnosis of previous myocardial infarction was made.

CASE 9 (A461768).—A man, aged forty-seven years, came to the Mayo Clinic with his wife, who was the patient. One morning while here, following an unusually heavy
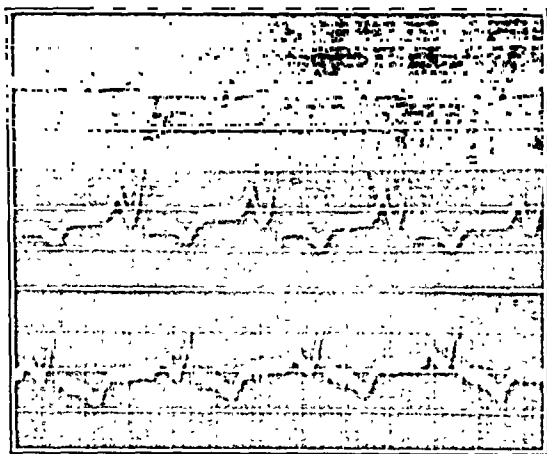

Fig. 24.—Case 9 (A461768). Taken day after tracing in Fig. 23. T wave negativity in Derivations II and III.
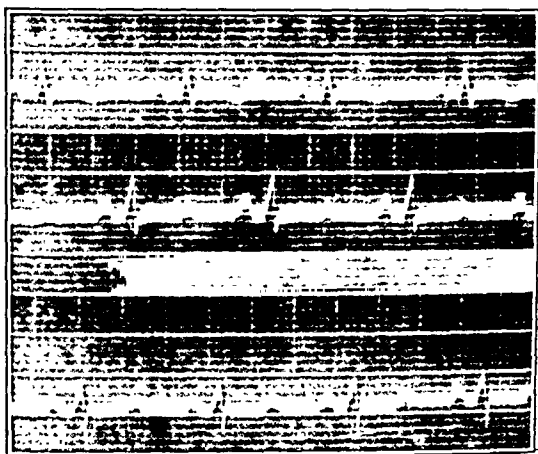
Fig. 25.—Case 9 (A461768). Taken four days after tracing in Fig. 24. T wave negativity in Derivation I.

breakfast, he suddenly developed a severe low substernal and high epigastric pain, radiating into the left arm. He was given 1/175 gr. of nitroglycerin without relief and was immediately sent to the hospital.

The general examination was essentially negative. The heart was not enlarged; there were no murmurs, but a slight respiratory arrhythmia. An electrocardiogram taken about fifteen minutes after his admission to the hospital revealed a sinus arrhythmia. (Fig. 21). The patient was given repeated injections of morphine hypodermically without relief. The attack subsided after seven hours. A diagnosis of coronary thrombosis was made.

The next day the electrocardiogram revealed occasional nodal premature contractions, but no significant abnormalities (Fig. 22). The heart was still objectively negative, but the leucocyte count was 12,600 and the temperature was 100.6°. The electrocardiogram, taken the next day, showed T wave negativity in Derivation I, with evidence of right bundle branch block, and was in all characteristics different from those on the preceding days (Fig. 23). Daily electrocardiograms were taken, but only the definite transitions will be recorded. On the next day the electrocardiogram revealed T wave negativity in Derivations II and III, and the curves were again markedly

different from those on the preceding day (Fig. 24). During this period the patient remained comfortable; there was no change in the objective heart findings; and it was not possible to elicit a pericardial rub. Four days later the electrocardiogram had again changed, the T wave being negative in Derivation I (Fig. 25). Nine days later the T wave was still negative in Derivation I, but showed greater amplitude (Fig. 26), and the electrocardiogram taken twelve days later was similar, but also showed ventricular premature contractions (Fig. 27). The last tracing, taken four days later, and the day of the patient's dismissal, was the same (Fig. 28). The heart at this time was slightly enlarged, the tones rather distant and without the clear-cut differentiation of the normal heart tones. The patient was up and about for part of the day, but responded poorly to slight cardiac overload.
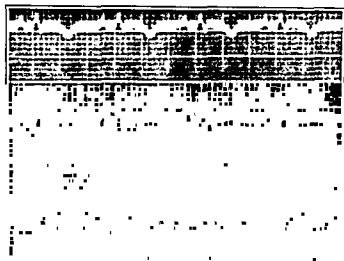
Fig. 26.—Case 9 (A461768). Taken nine days after tracing in Fig. 25. T wave negativity in Derivation I.

Fig. 27. — Case 9 (A461768). Taken twelve days after tracing in Fig. 26. T wave negativity in Derivation I. Ventricular premature contractions.

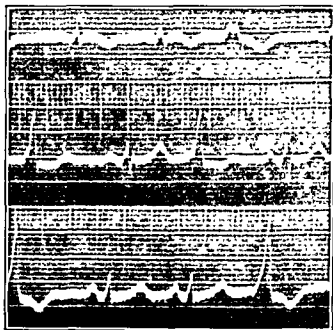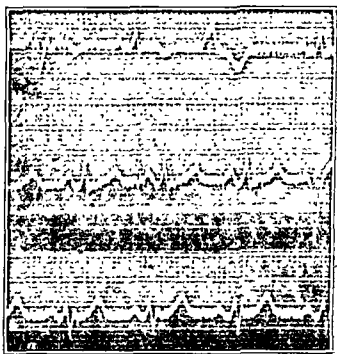Fig 28.—Case 9 (A461768). Taken four days after tracing in Fig 27. T wave negativity in Derivation I. Ventricular premature contractions

Five of the nine patients in this series died; postmortem examination was made in all.

DISCUSSION OF ELECTROCARDIOGRAMS

The most constant and striking changes occurring in the electrocardiograms were those affecting the T wave; such abnormalities occurred in eight

TABLE I

MYOCARDIAL INFARCTION (SERIES OF WILLIUS AND BARNES)

CASE	SEX AND AGE	TRAC-ING	ELECTROCARDIOGRAM	REMARKS
1 (A148902)	M 57	1	Taken about one-half hour before death, T wave negativity in Derivations I, II and III. Left ventricular preponderance	Necropsy. Infarct in the upper half of the interventricular septum
2 (A298068)	M 42	1	Taken three days after admission and about three and one-half weeks after history of occlusion. T wave negativity in Derivation I. Left ventricular preponderance	Necropsy. Hemorrhagic infarct of the apex of left ventricle
		2	Taken four days after first tracing and on the day preceding death. Nodal tachycardia, T wave negativity in Derivation I, left ventricular preponderance	
3 (A399583)	M 65	1	Taken three days after admission and twenty-three days before death. Sinus rhythm. Left ventricular preponderance	Necropsy. Infarct of left ventricle
(A401659)	M 70	1	Taken during attack. T wave negativity in Derivations I, II and III	Patient recovered
		2	Taken same day as first tracing. Sinoauricular block (only Derivation III taken)	
		3	Taken seven months after first tracing. T wave negativity in Derivation I, left ventricular preponderance	
5 (A42825)	M 53	1	Taken three days after admission and sixty days after attack. Auricular flutter, ventricular rate 83 to 169, auricular rate 327 to 338 (2:1 to 4:1 block)	Necropsy. Aneurysm of left ventricle following infarction. Left descending coronary occluded by thrombus
		2	Taken three days after first tracing. Nodal tachycardia	
		3	Taken two days after second tracing. Premature contractions. Tendency for fusion of R and T waves in Derivations I and II	
		4	Taken day after third tracing and on the day of death. Delayed A-V conduction (P-R 0.24 second). Probably effect of digitalis	
6 (A425425)	M 43	1	Taken two days after admission and before attack. T wave negativity in Derivations II and III	Patient recovered
		2	Taken during attack four days after first tracing. Tendency for fusion of R and T waves in Derivations II and III. T wave negativity in Derivations II and III	-- ---
		3	Taken day after second tracing. T wave negativity in Derivations I and II. T wave diphasic in Derivation III. Fusion of R and T waves has disappeared	
		4	Taken day after third tracing. T wave negativity in Derivations I, II and III	
		5	Taken day after fourth tracing. T wave negativity in Derivations I, II and III	

TABLE I—CONT'D

MYOCARDIAL INFARCTION (SERIES OF WILLIUS AND BARNES)

CASE	SEX AND AGE	TRACING	ELECTROCARDIOGRAM	REMARKS
7 (A448624)	M 67	1	Taken during attack. Ventricular premature contractions. Notched QRS complexes in Derivations I and II (QRS 0.12 second) T wave negativity in Derivations II and III	Necropsy. Multiple infarcts of the left ventricle
		2	Taken day after first tracing. Notched QRS complexes in Derivations I and II (QRS 0.12 second), T wave negativity in Derivations I, II and III	
		3	Taken two days after second tracing and two days before death. Notched QRS complexes in all Derivations (QRS 0.12 second). T wave negativity in Derivations I and II	
8 (A130266)	F 60	1	Taken forty-six days after attack. T wave negativity in Derivations I, II and III	Patient recovered
9 (A461768)	M 47	1	Taken during attack. Sinus arrhythmia	Patient recovered
		2	Taken day after first tracing. Nodal premature contractions	
		3	Taken day after second tracing. T wave negativity in Derivation I. Right bundle branch block	
		4	Taken day after third tracing. T wave negativity in Derivations II and III	
		5	Taken four days after fourth tracing. T wave negativity in Derivation I	
		6	Taken nine days after fifth tracing. T wave negativity in Derivation I. Greater amplitude of T wave	
		7	Taken twelve days after sixth tracing. T wave negativity in Derivation I. Ventricular premature contractions	
		8	Taken four days after seventh tracing and on day of dismissal T wave negativity in Derivation I. Ventricular premature contractions	

(89 per cent) cases (Table I). The most frequent change was the negativity of the T wave involving Derivation I, combined Derivations I and II, combined Derivations II and III, and combined Derivations I, II, and III. In three cases (Cases 4, 7 and 9), the derivations involved varied during the period of observation. In two cases (Cases 5 and 6) a tendency for fusion of the R and T wave occurred as a transient manifestation, and quite closely resembled the finding described by Pardee.

Notching and broadening of the QRS complex involving all derivations occurred in only one instance (Case 7), at first affecting only Derivations I and II, but ultimately involving all derivations.

In one case (Case 2) a paroxysm of nodal tachycardia occurred preceding death, and in another case (Case 5) auricular flutter and nodal tachycardia.

The electrocardiogram was normal in only one case (Case 3); it was taken twenty-three days before death and is, therefore, not representative, as possibly alterations would have been noted if other tracings had been obtained. The cases of other observers are recorded in Table II.

SUMMARY OF AGGREGATE SERIES

It is interesting to note that the T wave showed changes in twenty-six of the thirty-one cases (84 per cent, Table III). The alterations consisted of

TABLE II

MYOCARDIAL INFARCTION (CASES COLLECTED FROM THE LITERATURE)

AUTHOR	CASE	SEX AND AGE	TRAC-ING	ELECTROCARDIOGRAM	REMARKS
Levine and Trantor	2	M 59	1	Taken thirty-six hours after admission. Complete heart block, auricular rate 143, ventricular rate 29	Necropsy. Left anterior coronary artery was occluded by a thrombus
Herrick	1	M 42	1	Taken forty-two days after occlusion. Negative T wave in Derivations I and II Left ventricular preponderance	Necropsy. Descending branch of left coronary and first descending branch of the circumflex obstructed by a thrombus
			2	Taken 137 days after first tracing. Negative T wave in Derivation I. Left ventricular preponderance	
Pardee	1	M 38	1	Taken four hours after attack. Fusion of the R and T waves especially in Derivations II and III. T wave negative in Derivation I, positive in Derivations II and III	
			2	Taken three days after first tracing. Fusion of R and T waves absent. T wave negative in Derivation III	
			3	Taken six days after second tracing. T wave negative in Derivations II and III	
			4	Taken seven days after third tracing, same	
			5	Taken sixty-six days after fourth tracing, same	
Robinson and Herrmann		M 53	1	Taken soon after admission. Ventricular tachycardia, rate 185	Necropsy. Obstruction of anterior descending branch of the left coronary
			2	Taken ten hours after first tracing. Sinus rhythm, negative T wave in Derivation I	
Kahn	1	M 51	1	Taken fifty-one days after attack. Nodal premature contractions. Negative T wave in Derivation I, broadening of the QRS in all derivations, 0.12 second. Left ventricular preponderance	Necropsy. Nearly complete calcareous obstruction of the left coronary. An area of softening about 2 cm. in circumference at the apex of the left ventricle
			2	Taken sixty-seven days after first tracing and a few days before death. Diphasic T wave in Derivation I, QRS 0.10 to 0.12 second. Left ventricular preponderance	
	2	F 46	1	Taken thirty-nine days after occlusion. Negative T wave in Derivation I, notched QRS in Derivation II. Left ventricular preponderance	Necropsy. Occlusion of anterior descending branch of the left coronary
			2	Taken 113 days after first tracing, one day before death. Negative T wave in Derivation I, but less amplitude. Notched QRS in Derivation II, left ventricular preponderance more marked	

TABLE II—CONT'D

MYOCARDIAL INFARCTION (CASES COLLECTED FROM THE LITERATURE)

AUTHOR	CASE	SEX AND AGE	TRAC-ING	ELECTROCARDIOGRAM	REMARKS
Reznikoff	1	M 43	1*	Alternate slowing and acceleration of rate in units of about 8 beats. Interpreted by author as either a phenomenon of two foci, a normal sinus and a focus with a slower rate near the sinus, or an alternating bradycardia and tachycardia from the same focus	Necropsy. Thrombosis in the left coronary artery and an irregular laceration in the wall of the left ventricle in immediate proximity to the interventricular septum, midway between the apex and base
Wearn	1		1*	Taken four days after onset of attack and two days before death. Auricular premature contractions, negative T waves in Derivations II and III	
	2		1*	Taken two days after onset of attack and on the day of death. Complete heart block, QRS spread in all derivations, negative T wave in Derivation I, left ventricular preponderance	
	3		1*	Taken nine days after onset of attack and on the day of death. Ventricular premature contractions, T wave isoelectric in all derivations	
	4		1*	Taken fourteen days after onset of attack and ten days before death	
	5		1 to 5*	Rhythm at first irregular, delayed A-V conduction and complete heart block later. The QRS was normal on the fourth, twelfth and fourteenth days with marked lengthening on the sixth and seventh days. On the fourth day the T wave was diphasic in Derivation I, isoelectric in Derivation II and negative in Derivation III. On the seventh day the T wave was diphasic in Derivation I. On the fourteenth day the T wave was diphasic in Derivations I and II and isoelectric in Derivation III	Patient died on the fourteenth day
	6		1*	Taken three days after onset of attack and on the day preceding death. T wave isoelectric in Derivation III	
	7		1*	Taken day after onset of attack and on the day of death. Ventricular premature contractions, diphasic T wave in Derivation III. Left ventricular preponderance	
	8		1*	Taken one and two days after onset of attack and five and six days before death. Diphasic T wave in Derivation I. The T wave in Derivation II comes off the downstroke of R but on the following day this had returned to normal. Left ventricular preponderance	
	9		1*	Ventricular premature contractions. T wave isoelectric in all derivations, left ventricular preponderance	Onset of attack indefinite

TABLE II—CONT'D

MYOCARDIAL INFARCTION (CASES COLLECTED FROM THE LITERATURE)

AUTHOR	CASE	SEX AND AGE	TRAC-ING	ELECTROCARDIOGRAM	REMARKS
Wearn	10		1*	Taken twenty-nine days before death. QRS notched in all derivations. Diphasic T wave in Derivation I and negative in Derivations II and III, left ventricular preponderance	
Smith	1	M 44	1	Taken nineteen days after accident. Negative T wave in Derivations I, II and III. Right ventricular preponderance	Stab wound of heart and ligation of descending branch of left coronary artery in operative repair. Patient recovered
			2	Taken seventeen days after first tracing. Negative T wave in Derivations I, II and III but less amplitude than in first tracing. Right ventricular preponderance	
			3	Taken twenty-three days after second tracing. Negative T wave in Derivations I, II and III, less amplitude	
			4	Taken eight and one-half months after the accident. Diphasic T wave in Derivation III	
	2	M 51	1	Taken six hours after onset of attack. Negative T wave in Derivation I	The patient recovered
			2	Taken the day after first tracing. Negative T wave in Derivation I with much greater amplitude. The T wave in Derivation II almost isoelectric	
			3	Taken five hours after second tracing. Negative T wave in Derivations I and II	
			4	Taken day after third tracing. Negative T wave in Derivation I, small positive T wave in Derivation II	
			5	Taken day after fourth tracing, same	
			6	Taken day after fourth tracing, same	
	3	M 39	1	Taken twenty-four hours after onset of attack. Negative T wave in Derivation I	Patient died twenty-one days after occlusion. Necropsy. Occlusion of descending branch of left coronary and two small branches of circumflex
			2	Taken three days after first tracing. Diphasic T wave in Derivation III	
			3	Taken eleven days after second tracing. Negative T wave in Derivations I and II	
	4	M 42	1	Taken twelve days after attack. Negative T wave in Derivation III	Patient recovered
			2	Taken thirty-three days after first tracing. T wave isoelectric in all derivations	
			3	Taken eleven and one-half months after attack. T wave isoelectric in Derivation I	
	5	M 63	1	Taken three weeks after attack. Negative T wave in Derivations I, II and III	Necropsy. Descending branch of left coronary occluded
			2	Taken six months after first tracing. Negative T wave in Derivation I	
Nathanson 1923	1	M 64	1**	Taken during attack. Marked exaggeration of the T wave in all derivations with negativity in Derivations II and III. Left ventricular preponderance	
Riesman	2	M 45	1*	Irregularity in spread of excitation wave in ventricle and a curve compatible with coronary obstruction	

*Electrocardiograms not published but descriptions given.
**This case is reported through the courtesy of Dr. M. H. Nathanson of Minneapolis, under whose care the observations were made.

TABLE III

	CASES
Changes in the T wave (negative, diphasic and isoelectric curves) in Derivations I; I and II; II and III; I, II and III	26
Fusion of R and T waves	4
Complete heart block	3
Delayed A-V conduction	1
Aberrant QRS complexes in all derivations	5
Sinoauricular block	1
Ventricular tachycardia	1
Nodal tachycardia	2
Auricular flutter	1
Normal	3

negative, diphasic and isoelectric waves in Derivation I, combined Derivations I and II, combined Derivations II and III, and in combined Derivations I, II and III.

Fusion of the R and T wave occurred in four cases, and is such a definite alteration that its presence should always direct attention to the possible existence of acute coronary obstruction. This finding has also been observed during graphic study of the human heart at the time of death.

Complete heart block was observed in three cases. Aberration of the QRS complex in all derivations occurred in five cases. The other findings, delayed A-V conduction, sinoauricular block, ventricular tachycardia, nodal tachycardia and auricular flutter, occurred with such infrequency as to be unimportant. It is significant, however, that there are only three normal electrocardiograms (10 per cent).

The frequency of T wave changes in myocardial infarction we believe is significant, and these findings, in the presence of a suggestive clinical syndrome, will undoubtedly increase the frequency of correct clinical diagnosis. It is hoped that further reports of cases will appear in the literature, as a larger study will obviously be valuable.

BIBLIOGRAPHY

[1] Herrick, J. B.: Thrombosis of the coronary arteries. Jour. Am. Med. Assn., 1919, lxxii, 387-391.
[2] Kahn, M. H.: Aneurysm of the left ventricle. Am. Jour. Med. Sc., 1922, clxii, 839-854.
[3] Levine, S. A., and Trantor, C. L.: Infarction of the heart simulating acute surgical abdominal conditions. Am. Jour. Med. Sc., 1918, clv, 57-66.
[4] Pardee, H. E. B.: An electrocardiographic sign of coronary artery obstruction. Arch. Int. Med., 1920, xxvi, 244-258.
[5] Reisman, D.: Coronary thrombosis, with an account of the disease in two brothers and remarks upon diagnosis and treatment. Med. Clin. N. Amer., 1923, vi, 861-871.
[6] Reznikoff, P.: The auscultatory sign in a case of spontaneously rupturing heart. Jour. Am. Med. Assn., 1922, lxxviii, 1296.
[7] Robinson, G. C.: A study with the electrocardiograph of the mode of death of the human heart. Jour. Exper. Med., 1912, xvi, 291-303.
[8] Robinson, G. C., and Herrmann, G. R.: Paroxysmal tachycardia of ventricular origin, and its relation to coronary occlusion. Heart, 1921, viii, 59-81.
[9] Smith, F. M.: Electrocardiographic changes following occlusion of the left coronary artery. Arch. Int. Med., 1923, xxxii, 497-510.
[10] Wearn, J. T.: Thrombosis of the coronary arteries, with infarction of the heart. Am. Jour. Med. Sc., 1923, clxv, 250-276.
[11] Willius, F. A.: Changes in the mechanism of the human heart preceding and during death. Med. Jour. and Rec., 1924, cxix, (Symposium on the cardiovascular system) xlix-liv.

In view of making the comparison more marked, the information obtained from this data is given in the curves of Type III together with the results of analogous experiments made on the same material during its alkalinization. Plate III-C illustrates the conductance changes in a solution of arsphenamine C during its alkalinization and subsequent acidification back to the dihydrochloride. Inasmuch as a break in the alkalinization curve had occurred after about 2.8 c.c. 0.1 N NaOH had been added per 0.1 gm. sample
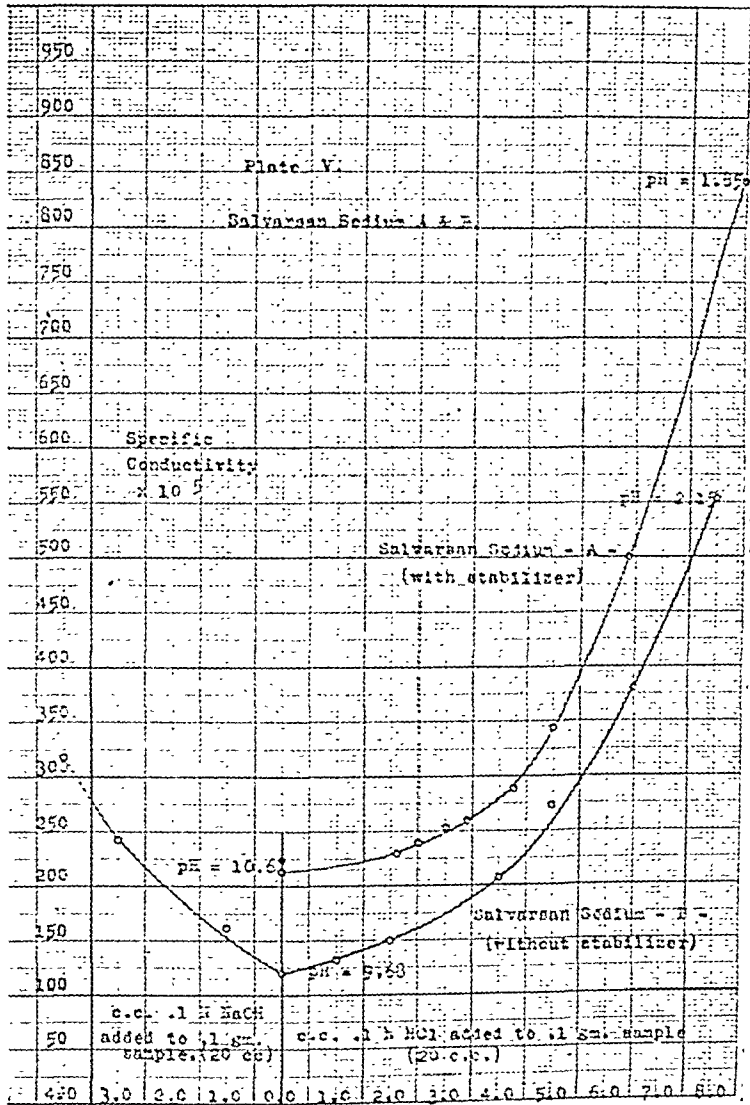
Plate V.

of the dihydrochloride, it might be expected that a similar break might occur after about 5.7 c.c. 0.1 N HCl had been added to the same alkalinized solution. Such was not the case. Instead, a minimum point did occur in the case of arsphenamine C when 1 c.c. 0.1 N HCl had been added; and, from that point the conductance increased continuously. That this phenomena was general is evidenced by Plate III-K which gives the results of the experiments conducted on the six different commercial preparations examined. It must be noticed that in the cases of arsphenamines A, B, C, G, and I that

the break came when 1.0 c.c. acid had been added, while with arsphenamines D, E, F and H the minimum was found only after 2.0 c.c. acid had been added. Table XV shows the relation between the increases in conductance of the different preparations upon alkalinization and acidification.

Plate III-C.

Plate III-K.

TABLE V

ARSPHENAMINE C (ACIDIFICATION OF ALKALINIZED SOLUTION)
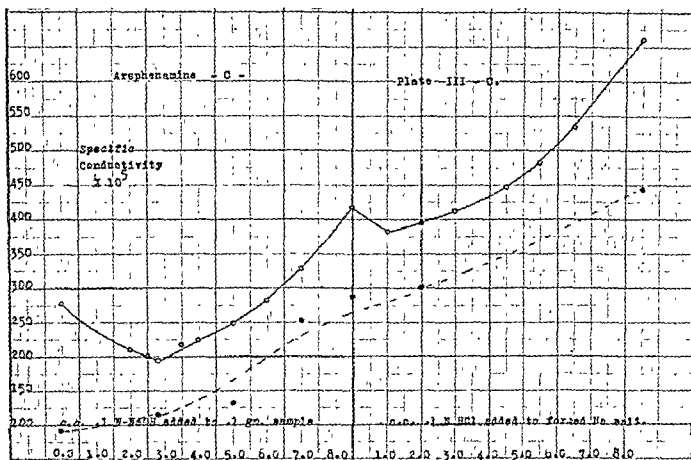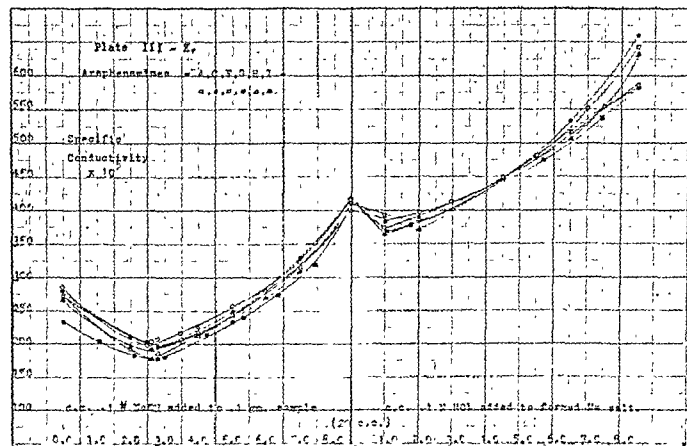
EXP.	C.C. 0.1 N NaOH PER 0.1 GM.	C.C. 0.1 N HCL PER 0.1 GM.	SP. C. X 10⁴	VOLTS	P_H
245	8.5	0.0	41.78	0.9288	11.52
254	8.5	1.0	38.23	0.9012	11.05
255	8.5	2.0	39.50	0.8834	10.75
256	8.5	3.0	41.25	0.8517	10.22
257	8.5	4.5	44.74	0.6826	7.356
258	8.5	5.5	48.31	0.4691	3.743
259	8.5	6.5	53.46	0.4270	2.475
260	8.5	8.5	66.11	0.3941	3.032

Column 2 gives the NaOH used to alkalinize the sample of the dihydrochloride; Column 3, the HCl used in acidification; Column 4, the specific conductivity of the solution; Column 5, potentiometric reading in volts (25° C.) and Column 6, the equivalent P_H values.

TABLE XV

COMPARISON OF SPECIFIC CONDUCTIVITY CHANGES

ARSPHENA-MINE	DIHYDRO-CHLORIDE	DISODIUM	INCREASE	DIHYDRO-CHLORIDE	INCREASE	TOTAL INCREASE
A	28.46	41.65	13.19	64.22	22.57	30.76
B	28.43	34.63	10.39	60.06	21.24	31.63
C	27.82	41.78	13.96	66.11	24.33	38.29
D	29.97	42.41	12.30	64.79	22.52	34.82
E	30.82	42.44	11.62	65.01	22.57	34.19
F	27.01	41.03	14.02	58.48	17.45	31.47
G	23.20	41.24	18.04	58.40	17.16	35.20
H	27.19	39.93	12.74	64.49	24.56	37.30
I	26.88	41.96	15.08	63.40	21.44	36.52

Column 2 gives the specific conductivity of the dihydrochloride solution; column 3, that of the alkalinized solution; column 4, increase upon alkalinization; column 5, specific conductivity of solutions reconverted into the dihydrochloride; column 6, increase due to reconversion and column 7, total increase in going from dihydrochloride to dihydrochloride.

TABLE XVI

COMPARISON OF P_H CHANGES

ACIDIFICATION OF THE ALKALINIZED SOLUTION

SAMPLE	C.C. 0.1 N HCL ADDED PER 0.1 GM. SAMPLE										C.C. 0.1 N HCL AT MINIMUM
	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	8.5	
A	11.55	11.22	10.51	10.03	8.35	4.90	3.58	2.97	2.97	2.52	1.0
B	11.40	11.16	10.78	10.10	8.80	4.60	3.40	2.92	2.64	2.46	1.0
C	11.52	11.15	10.75	10.22	8.75	4.78	3.40	2.90	2.60	2.47	1.0
D	11.56	11.12	10.74	10.09	9.34	4.65	3.90	2.92	2.60	2.42	2.0
E	11.52	11.22	10.69	9.96	9.23	5.30	3.40	2.94	2.70	2.60	2.0
F	11.51	10.89	10.45	10.07	8.85	4.50	3.40	2.95	2.68	2.58	2.0
G	11.42	10.82	10.45	10.12	9.60	7.00	3.55	2.98	2.68	2.56	1.0
H	11.54	11.25	10.54	9.99	9.18	5.35	3.58	3.00	2.70	2.61	2.0
I	11.56	11.27	10.82	10.09	8.70	5.05	3.48	3.00	2.70	2.60	1.0

When a comparison is made of the P_H values of the respective solutions during alkalinization and subsequent acidification no differences occur. The curves of Type IV show that the change in the hydrogen-ion concentration of the solutions during the acidification of the alkalinized solution is almost identical but reversed from that change which occurred during the alkalinization. Plate IV-C shows the two sides of the curve to be mirror images of each other. This is of course in accord with theory. Plate IV-K shows that this same relationship holds for the commercial preparations. Arsphenamine

G seems to be somewhat different from the rest in that it exhibits a sharper break at the point of alkalinization.

Table XVI gives a comparison of the P_H changes of the respective solutions during acidification. Although some difference occurs in the middle of the precipitation region, it is not as great a variation as was found in a similar spot during alkalinization. (Part I.) The figures show that as far as chemical reaction goes there is no difference between that occurring in the acid solution or the alkaline solution. On the other hand, the difference in the conductance of the two solutions illustrates most strongly a difference in the physical condition of the arsphenamine molecule within the solutions.
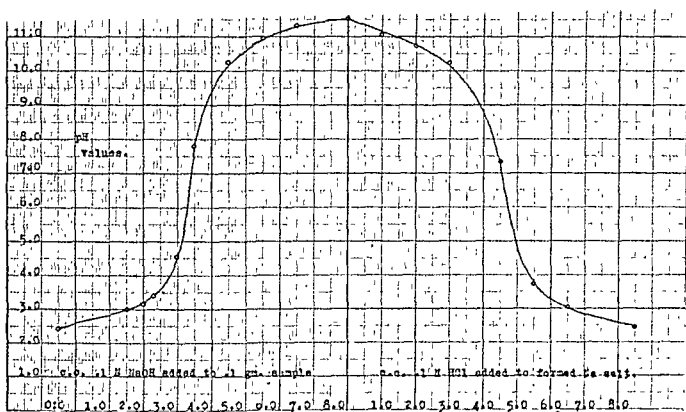

Plate IV-C

It is to this physical condition that the cause of the toxicity and therapeutic activity must be traced.

In view of the fact that an isotonic saline is recommended by certain clinics (Fordyce, Rosen and Myers, 1923) as a diluent of an alkalinized solution to be used for intravenous injection, it was thought worth while to examine an isotonic arsphenamine solution in order to determine the effect of the electrolyte on the behavior of the arsphenamine molecule in solution. A series of solutions were made up in the regular manner from arsphenamine C and to each the equivalent of 0.85 gm. NaCl was added per 100 c.c solution. The specific conductivity of 0.85 gm. NaCl in 100 c.c. was determined and taken as a blank. This value when subtracted from the conductances of the respective isotonic arsphenamine solutions gave the conductance of the arsphenamine as such. Now, if these values for the conductance of the arsphenamine, when plotted in the usual manner against

the alkali and acid used, showed any marked deviation from the original curve, such a divergence must have been due to the effect of the excess of electrolyte. Although only preliminary experiments have been completed, a divergence was noted. No minimum was found in the entire curve. Instead, the conductance values increased throughout the alkalinization as well as the acidification.

The conductance of the arsphenamine itself is indicated roughly by the dotted curve on Plate III-C. This abnormal conductivity throughout the entire curve suggests the presence of the colloidal electrolyte in the acid as well as the alkaline solution. Also, macroscopically there was a difference in the solutions which contained a precipitate. There the precipitate is non-
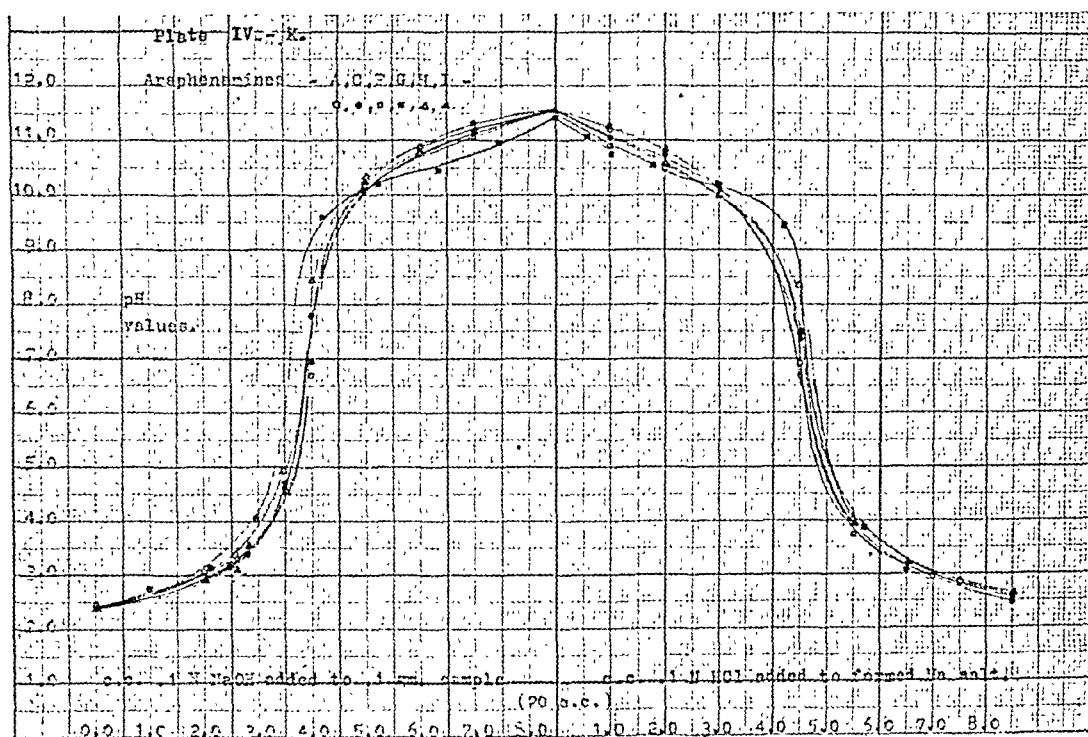

Plate IV-K.

sticky and it tends to form small dense particles in contrast to the gelatinous nature of usual precipitates. This would be expected from colloidal generalizations.

It was observed that upon standing, a sealed, alkalinized solution slowly acquired a red coloration; it became turbid and finally there settled a red gel leaving a colorless supernatant liquor. Gel formation under these conditions required a period of at least three weeks. An attempt was made to ascertain the course of this transformation by following it with conductivity and hydrogen-ion measurements. For this purpose a solution of alkalinized arsphenamine was sealed in 50 c.c. Erlenmeyer flasks which were kept at 25° in an air bath. Plate VI shows how these respective factors changed with time. PH values dropped slightly, a fact which could not be in accord with an hydrolysis of the sodium salt and the deposition of the base as the

red gel. It was observed that if a turbid solution was exposed to the air for a few minutes and then resealed, that the final supernatant liquor had a green coloration, a phenomena which is often noticed in a solution made up of dry salvarsan sodium. Doubtless, this change is due to some structural polymerization as atmospheric oxidation results in a brownish black residue.

The irregularity of the conductivity curve, the deviations of which are beyond the experimental error, merely emphasizes the complexity of the re-arrangement. The turbid solutions poisoned the electrodes and consequently measurements could not be obtained beyond that point.

At present it does not seem possible to correlate the various minor discrepancies which have appeared in the different phases of this investigation. Although it is clearly recognized that the disturbing influences such
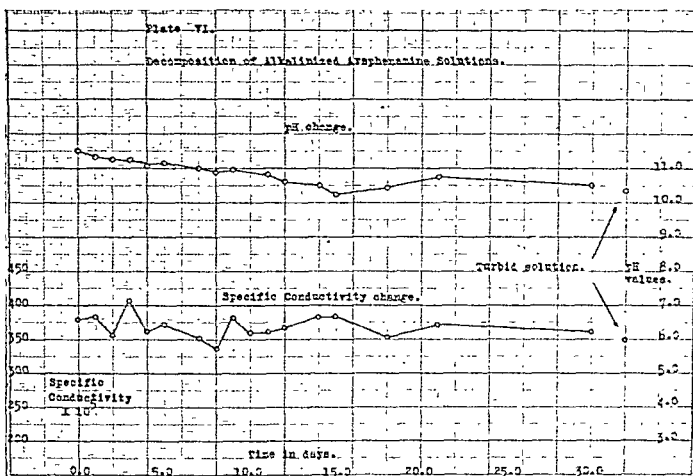
Plate VI

as hydration, polymerization, etc., might affect the position of the minimum conductivity and consequently shed uncertainty upon the indicated position of the isoelectric point, the fact that the found minimum has been so consistent tends to show that these outside effects have been negligible. Among the arsphenamines examined were preparations of French, German, and American manufacture as well as materials which were very soluble together with those difficultly so. It would seem that if the possible disturbing influences were to make themselves felt that they would have shown up clearly among such an assortment.

The question of the abnormal conductivity merits study, as it is a characteristic phenomena exhibited by the so-called colloidal electrolytes. McBain (1922) has applied the principle of a colloidal electrolyte to soap solutions

which also possess an abnormal conductivity. Soap solutions according to this idea, are supposed to undergo an ionization which might be expressed by the following equilibrium.

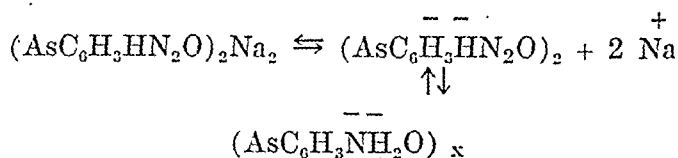$$RNa \rightleftarrows \overset{-}{R} + \overset{+}{Na}$$
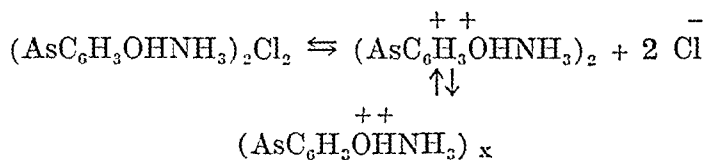$$\uparrow\downarrow -$$
$$(R)_x$$
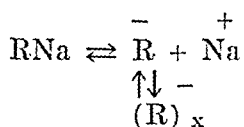
where the dissociated fatty acid ion R is also in equilibrium with an aggregate of itself which possesses an accumulated charge. This aggregate is termed "ionic micelle" and to it, by virtue of its accumulated charge, is attributed the abnormal conductivity. In the case of soap, i.e., the salts of fatty acids, true solution exists only in great dilutions under which condition little or none of the ionic micelle form is present. Only in such cases do soap solutions show a normal conductance.

Now consider this principle as applied to arsphenamine solutions: The ionization of the sodium salt would be:

$$(AsC_6H_3HN_2O)_2Na_2 \leftrightarrows (AsC_6\overset{-}{H}_3\overset{-}{HN}_2O)_2 + 2\ \overset{+}{Na}$$
$$\uparrow\downarrow$$
$$(AsC_6\overset{-}{H}_3\overset{-}{NH}_2O)_x$$

On the other hand, the ionization of the dihydrochloride might be expressed:

$$(AsC_6H_3OHNH_3)_2Cl_2 \leftrightarrows (AsC_6\overset{+}{H}_3\overset{+}{OHNH}_3)_2 + 2\ \overset{-}{Cl}$$
$$\uparrow\downarrow$$
$$(AsC_6\overset{+}{H}_3\overset{+}{OHNH}_3)_x$$

This investigation has shown that in the solutions studied, abnormal conductivity, indicating the presence of the ionic micellae of the colloidal electrolyte, became manifest at the P_H of about 10.5. At that hydrogen-ion concentration the anion $(AsC_6H_3NH_2O)$ must be present to some extent in the form of the ionic micelle. Plates III (A-K) show that the conductivity increased in an abnormal manner after the addition of 1.0 or 2.0 c.c. 0.1 N HCl had been added per 0.1 gm. sample to the alkalinized solution. It is possible to conceive that just at those points a sufficient concentration of Na-ions has been built up, due to the NaCl formed by metathesis, to suppress the normal ionization which instead of resulting in the increase of molecular arsphenamine, causes a building up of the ionic micellae. On the other hand, in the isotonic solutions, where there is an excess of the neutral electrolyte present at all times, the ionic micellae are built up at the expense of the true ions. Consequently, instead of a decrease in the conductance upon alkalinization of the hydrochloride there is found the constant increase.

The presence of ionic micellae in arsphenamine solutions might prove an explanation for the paradox presented by the work of Voegtlin and Johnson (1924) on the relationship between the viscosity and the toxicity of the drug.

They found in the hydrochloride solutions that the most viscous were the most toxic while upon alkalinization the most viscous hydrochlorides became the least toxic. This difference in the acid and alkaline solutions could not be explained.

This investigation has pointed out that the colloidal electrolyte, i.e., the ionic micelle form, as indicated by the abnormal conductivity did not make itself manifest in the hypertonic solutions until after such a solution had been alkalinized and its P_H lowered by the addition of acid. A similar drop in the P_H of the alkalinized solution must occur when the solution is injected intravenously, due to the buffer action of the blood. The alkalinized solution then contains the ionic micelle form of the drug. On the other hand, Plates II (A–K) show that as the alkalinization of the hydrochloride proceeds and the P_H value increases, that the conductivity drops suddenly into a region which is characterized by the molecular form of the drug and a subsequent absolute precipitation of the base before the P_H of the blood stream is reached. Undoubtedly the extreme toxicity of the dihydrochloride and the monohydrochloride is because of the fact that, due to the buffer action of the blood, such solutions must pass through this point of minimum conductivity where for the most part only the molecular base can stay in solution. Such a condition would lend itself most readily to the formation of emboli which are always to be found following a fatality caused by such an injection. Undoubtedly, the aggregated ion would be in a physical state much more suitable for its union, possibly with some protein in the blood stream than would be any molecular form of the base. Such a combination would favor longer retention and would lessen the tendency for direct precipitation by electrolytes.

SUMMARY

1. A study of the alkalinized solution of arsphenamine has been made with the purpose of obtaining information as to its physical and colloidal nature.

2. The conductance of salvarsan sodium solutions does not drop to a minimum when the solutions are acidified to the dihydrochloride; instead it constantly increases.

3. When freshly alkalinized solutions are acidified, the conductivity measurements show a minimum after 1.0 or 2.0 c.c. of 0.1 N HCl have been added per 0.1 gm. sample. No minimum occurs at a P_H value corresponding to that of the isoelectric point found during the alkalinization of the dihydrochloride.

4. P_H measurements show that the changes in the solution upon the acidification of an alkalinized solution are identical and the reverse of those occurring during the alkalinization.

5. Conductance measurements, on the other hand, show that there is a physical difference between the acid and alkaline solutions of arsphenamine. The abnormal conductivity shown in the latter indicates that arsphenamine is a colloidal electrolyte.

6. Observations were made upon the changes which take place in an alkalinized solution upon standing.

7. An isotonic arsphenamine solution shows no minimum conductance either upon alkalinization or subsequent acidification.

8. An explanation is offered for the abnormalities found in the conductivity measurements in the different solutions which is based upon McBain's conception of an ionic micelle found in solutions of colloidal electrolytes. This offers a possible explanation of the differences between the toxicities of the acid and alkaline solutions.

REFERENCES

[1] Aubry, A. and Dermay, E.: Diglucosidediaminodihydroxydiarsenobenzene, Compt. rend., Acad. d. sc., 1922, clxv, 819-822.
[2] Fordyce, J. A., Rosen, I., and Myers, C. N.: Quantitative Studies in Syphilis from a Clinical and Biological Point of View. IV. The Arsenic Content of the blood at various Intervals after Intravenous Injection of Salvarsan. Am. Jour. Syph., 1923, vii, 225.
[3] McBain, J. W.: The Study of Soap Solutions, Rep. Brit. Assn., Adv. Sci. Lond., 1923, i, 306.
[4] Voegtlin, C., Johnson, J. M., and Dyer, H.: Viscosity and Toxicity of Arsphenamine Solutions, Pub. Health Rep., 1923, 898.

A PHARMACODYNAMIC STUDY OF THE ANTHELMINTIC PROPERTIES OF TWO OILS OF CHENOPODIUM[*]

By A. Richard Bliss, Jr., A.M., Phm.D., M.D., Memphis, Tenn.

INTRODUCTION

THESE investigations were prompted by the facts that (1) the supply of oil of Chenopodium ("American Oil of Wormseed") is inadequate for human and animal medication, and (2) the resulting high price of the "Maryland Oil of Chenopodium" is consequently preventing the extensive use of the drug in the treatment of domestic animals, a field in which it is much needed.[1]

In 1854 an article by Garrigues[2] described the Southern (U.S.) and the Western (U.S.) Chenopodium plants, and accredited the oils distilled from both varieties with equal anthelmintic properties. During the same year, very shortly after the appearance of this article, another paper[3] was published by a second writer protesting against Garrigues' statements concerning the efficacy of the oil distilled from the Western plants ("Western Oil of Chenopodium"), but offering no proofs of the superiority of the oil distilled from the Southern plants ("Maryland, Baltimore, or Southern Oil of Chenopodium").

The second article referred to is probably responsible for the generally accepted opinion that oil of Chenopodium, conforming to the requirements of the United States Pharmacopoeia,[4] can be produced only from *Chenopodium ambrosioides, var. anthelminticum* cultivated in Carroll County, Maryland. The work of Wirth[5] on an oil distilled from plants grown at the University of

Michigan Botanical Gardens apparently substantiated this opinion. The conclusion of Wirth and others, that the inferiority of the Western oil of Chenopodium was due to a low Ascaridol content, was apparently based entirely upon the low specific gravity of the Western oil they had obtained.

Schimmel's reports[6] indicate that Ascaridol, which apparently corresponds to the heavier fraction obtained in the distillation of oil of Chenopodium, is the part responsible for the anthelmintic properties of the drug. Although this statement has not been proved and no reasons for the conclusion have been stated, it has been generally accepted. Hall and Hamilton[7] demonstrated that although Ascaridol is anthelmintic, it is also a very active gastrointestinal irritant; while the lighter fraction of the oil is more anthelmintic and decidedly less irritating. Salant and Nelson[8] found Ascaridol 30 per cent more toxic than oil of Chenopodium.

The reports of Schimmel[9] and the investigations of Nelson[10] and of Russell[11] indicate that the failure of Wirth and other workers to obtain a satisfactory oil from plants cultivated in the Middle West was probably due to the faulty methods of distillation employed. Konantz's[12] work substantiates the foregoing statement, for the oils prepared by him from Chenopodium plants cultivated in the Middle West meet the U. S. pharmacopeial specifications.

Although a considerable number of pharmacodynamic investigations of the anthelmintic properties of oil of Chenopodium ("Maryland Oil") have been carried out (particularly by Hall, Hamilton, Widger, Foster, Wilson, and Salant and coworkers), and while it is probably true that some of the oils used in these investigations were admixtures of the Maryland and the Western oils, up to the present time no pharmacodynamic studies have been made of the pure, unmixed Western oil or of the oil distilled from the wild Chenopodium of the Middle Western states.

MATERIALS AND METHOD

My pharmacodynamic investigations were carried out on three samples of oil of Chenopodium distilled by Konantz.[13] One of the samples was an oil (S/G 0.966*) distilled from plants which had been shipped from Carroll County, Maryland (referred to in this study as "Maryland Oil of Chenopodium"); a second was a sample of an oil (S/G 0.970) distilled from plants cultivated in Adams County, Illinois, (called "Western Oil of Chenopodium" in this article); and the third was a sample of an oil (S/G 0.964) derived from cultivated wild wormseed plants found in Adams County, Illinois, (the "Wild Western Oil of Chenopodium" hereafter referred to).

Because of the fact that oil of Chenopodium in single therapeutic dose has a decidedly higher efficacy against ascarides than is shown by any other anthelmintic, the common ascaris of the dog was used as the test worm in determining the anthelmintic value of the three oils studied. Other varieties of worms found in the experimental animals were disregarded. Hall and Foster,[14] and Hall[15, 16] established the superiority of oil of Chenopodium as an

ascaricide in dogs. Their results showed that the oil has no equal for the removal of ascarides, since it will, in the vast majority of cases, remove 100 per cent of these worms present in the dog. Clinical observations have shown that the oil is apparently about as effective, when properly administered, against ascarides in man. Hall and Foster[17] experimentally established its efficacy against ascarides in swine; Hall, Wilson and Wigdor[18] in the horse; and Hall[19] in the cat.

The method employed by the writer was that of Hall and Foster,[20] i.e., the collection of all worms from the feces after the treatment and from the dog postmortem. The standard dose of oil of Chenopodium used throughout the investigations was 0.1 mil per kilo, the amount conclusively established by Hall[21] as being capable of removing all ascarides from the dog in the great majority of cases. The apparatus employed was that advocated by Hall[22] for examining feces for evidences of parasitism. The oils were administered in soft, elastic capsules[23] according to the practical dosage recommended by Hall:[24] viz., 5 minims for dogs weighing ten pounds or less; 10 minims for dogs weighing ten to twenty pounds; 15 minims for dogs weighing twenty to thirty pounds; not to exceed 20 minims for dogs weighing over thirty pounds. One fluidounce (30 mils) of castor oil was given immediately after the oil of Chenopodium. The investigations of Hall and Wigdor[25, 26] and Hall and Hamilton[27] indicate that the administration of castor oil immediately after the oil of Chenopodium is of extreme importance, since it is protective and not merely purgative. Oil of Chenopodium is decidedly

TABLE I

WESTERN OIL OF CHENOPODIUM

NO.	DOG NO.	WT. IN KILOS	OIL CHEN. IN MINIMS	CASTOR OIL IN MILS	NO. ASCARIDES IN STOOLS	NO. ASCARIDES POSTMORTEM	DIGEST. TRACT	EFFICACY AGAINST ASCARIDES	NO.
1	1	10	15	30	6	0	Normal	100%	1
2	2	13	15	30	10	0	Normal	100%	2
3	3	13	15	30	7	0	Normal	100%	3
4	6	16	20	30	9	0	Normal	100%	4
5	7	15	20	30	18	0	Normal	100%	5
6	9	6	10	30	11	0	Normal	100%	6
7	10	12	15	30	4	0	Normal	100%	7
8	11	11.5	15	30	7	1	Normal	87.5%	8
9	14	8	10	30	21	0	Normal	100%	9
10	15	14	15	30	2	0	Normal	100%	10
11	16	14.5	20	30	8	0	Normal	100%	11
12	19	7.5	10	30	2	0	Normal	100%	12
13	21	12	15	30	9	1	Normal	90%	13
14	23	14.5	20	30	14	0	Sl. Inflam.	100%	14
15	24	15	20	30	35	0	Normal	100%	15
16	25	15	20	30	1	0	Sl. Inflam.	100%	16
17	27	12	15	30	23	0	Normal	100%	17
18	29	9	10	30	8	0	Normal	100%	18
19	31	15	20	30	6	0	Normal	100%	19
20	32	9.5	10	30	2	0	Normal	100%	20
21	33	12	15	30	10	2	Normal	83%	21
22	35	16	20	30	29	0	Sl. Inflam.	100%	22
23	38	6	10	30	5	0	Normal	100%	23
24	39	8	10	30	16	0	Normal	100%	24

Note 1: Average efficacy against ascarides, 98.35 per cent.
Note 2: Percentage showing slight inflammation, 12.5 per cent.

poisonous, constipating, and a gastrointestinal irritant. Castor oil retards the absorption of the oil of Chenopodium, distributes it over a larger surface of the gastrointestinal mucosa, and promotes elimination. My own observations substantiate the foregoing statements. Salant and Nelson[28] also demonstrated that certain fixed oils are of value in preventing poisoning by oil of Chenopodium.

Each experimental dog, housed in a separate, thoroughly cleaned cage, was given no solid food for twenty-four hours. Early the following morning

TABLE II
WILD WESTERN OIL OF CHENOPODIUM

NO.	DOG NO.	WT IN KILOS	OIL CHEN. IN MINIMS	CASTOR OIL IN MILS	NO. ASCARIDES IN STOOLS	NO. ASCARIDES POSTMORTEM	DIGEST. TRACT	EFFICACY AGAINST ASCARIDES	NO.
1	42	6	10	30	2	0	Normal	100%	1
2	43	7.75	10	30	8	0	Normal	100%	2
3	44	10	15	30	3	0	Normal	100%	3
4	45	16	20	30	18	2	Sl. Inflam.	90%	4
5	48	15	20	30	4	0	Normal	100%	5
6	51	8	10	30	16	0	Normal	100%	6
7	52	10.5	15	30	26	2	Normal	93%	7
8	54	11.5	15	30	11	0	Normal	100%	8
9	55	16	20	30	4	0	Sl. Inflam.	100%	9
10	56	17	20	30	1	0	Normal	100%	10
11	59	6.5	10	30	8	0	Normal	100%	11
12	62	6.75	10	30	2	0	Normal	100%	12
13	64	9.5	10	30	1	0	Normal	100%	13
14	67	10	15	30	20	0	Sl. Inflam.	100%	14
15	68	4.5	5	30	6	0	Normal	100%	15
16	69	6	10	30	5	0	Normal	100%	16
17	71	13.5	15	30	8	2	Normal	80%	17
18	72	12	15	30	12	0	Normal	100%	18

Note 1:   Average efficacy against ascarides, 97.77 per cent.
Note 2:   Percentage showing slight inflammation, 16.66 per cent.

TABLE III
MARYLAND OIL OF CHENOPODIUM

NO.	DOG NO.	WT IN KILOS	OIL CHEN. IN MINIMS	CASTOR OIL IN MILS	NO. ASCARIDES IN STOOLS	NO ASCARIDES POSTMORTEM	DIGEST. TRACT	EFFICACY AGAINST ASCARIDES	NO.
1	73	12	15	30	22	0	Normal	100%	1
2	74	15	20	30	12	0	Sl. Inflam.	100%	2
3	77	6	10	30	3	0	Normal	100%	3
4	79	7.5	10	30	5	0	Normal	100%	4
5	80	8.5	10	30	16	0	Normal	100%	5
6	81	10	15	30	8	0	Normal	100%	6
7	82	16	20	30	6	0	Sl. Inflam.	100%	7
8	84	15.5	20	30	1	0	Normal	100%	8
9	86	14.5	20	30	10	2	Sl. Inflam.	90%	9
10	87	9	10	30	2	0	Normal	100%	10
11	88	8	10	30	9	0	Normal	100%	11
12	90	12.5	15	30	6	0	Normal	100%	12
13	91	11.75	15	30	8	2	Normal	80%	13
14	92	6	10	30	3	0	Normal	100%	14
15	94	7	10	30	8	0	Normal	100%	15
16	95	10	15	30	9	0	Normal	100%	16
17	96	13.5	15	30	19	0	Normal	100%	17
18	98	7.5	10	30	3	0	Normal	100%	18
19	102	12	15	30	2	0	Normal	100%	19
20	104	15	20	30	30	0	Normal	100%	20

Note 1:   Average efficacy against ascarides, 98.5 per cent.
Note 2:   Percentage showing slight inflammation, 15 per cent.

the oil of Chenopodium and the castor oil were administered. No food was given for at least three hours after the drugs. All feces passed during the following five days were carefully examined for ascarides. At the end of the fifth day the animal was killed (shot), and the alimentary tract from the esophagus to anus was slit and examined for ascarides and lesions.

The results of the study are tabulated in Tables I, II, III, and IV.

SUMMARY

In Table I there are twenty-four dogs with a total of 267 ascarides; an average of 11 + per dog. Of these worms the treatment with the Western oil of Chenopodium removed 263. The treatment was, therefore, 98.50 per cent effective against ascarides, a value extremely close to the mathematical average efficacy in Note 1, Table I.

There are eighteen dogs in Table II with a total of 161 ascarides; an average of 9 – per dog. The treatment with the wild Western oil of Chenopodium removed 155 worms, and was, therefore, 96.27 per cent effective, which is a little less than the mathematical average efficacy in Note 1, Table II.

Table III shows twenty dogs with a total of 192 ascarides; an average of 9.6 per dog. The Maryland Oil of Chenopodium treatment removed 188 of the parasites, and was consequently 97.91 per cent effective, a small fraction less than the mathematical average efficacy in Note 1, Table III.

The foregoing figures are tabulated in Table IV.

TABLE IV

NO.	OIL	NO. OF DOGS	NO. ASCARIDES IN STOOLS	NO. ASCARIDES POSTMORTEM	PER CENT EFFICACY	NO.
1	Western Oil	24	263	4	98.50	1
3	Wild Western Oil	18	155	6	96.27	2
2	Maryland Oil	20	188	4	97.91	3

The average efficacy of the three samples of oil was, therefore, 97.56 per cent.

Examination of the digestive tracts of the animals showed a slight degree of inflammation in three dogs of each of the three series, or a total of nine animals out of sixty-two. This small percentage (14.5 per cent) and the very mild degree of inflammation found indicates that this possible phase of the action of the oils of Chenopodium, in the dosage employed, may be disregarded in the cases of all three oils unless the animal already shows a gastroenteritis. It is interesting to note that practically all the cases showing inflammation were among the heavier dogs which had received a larger dose of the oil. The reader is referred to Hall's study[29] of the lesions due to agents used in killing experimental dogs in anthelmintic investigations.

CONCLUSIONS

This study has shown that oil of Chenopodium properly distilled from plants cultivated in the Middle Western states is as efficacious in dogs against ascarides as the Maryland oil of Chenopodium. On the basis of the experiments with dogs I venture to state that these findings apply also to man.

I am indebted to W. A. Konantz of Quincy, Ill., for the samples of the oils used in this study; and also to Miss Pearl Waddell, A.B., and to C. H. Menge, A.B., for valuable technical assistance rendered during the investigations.

REFERENCES

[1] Konantz: Jour. Am. Pharm. Assn., March, 1924, xiii, No. 3, pp.201-205.
[2] Garrigues: Am. Jour. Pharm., September, 1854, xxv, 404-408.
[3] Am. Jour. Pharm., November, 1854, xxvi, 503.
[4] U. S. P., ix, 290.
[5] Wirth: Jour. Am. Pharm. Assn., September, 1920, ix, 127.
[6] Schimmel and Co.: Semi-An. Rept., April, 1908, p. 109.
[7] Hall and Hamilton: Jour. Pharmacol. and Exper. Therap., April, 1918, ix, No. 3.
[8] Salant and Nelson: Am. Jour. Physiol., 1915, xxxvi, 440.
[9] Schimmel and Co.: Report, April, 1908, p. 109.
[10] Nelson: Jour. Am. Chem. Soc., 1920, xlii, 1204.
[11] Russell: Jour. Am. Pharm. Assn., November, 1922, xi, 255.
[12] Konantz: Jour. Am. Pharm. Assn., March, 1924, xiii, No. 3, pp. 201-205.
[13] Konantz: Jour. Am. Pharm. Assn., xiii, No. 3, p. 447.
[14] Hall and Foster: Jour. Agric. Research, February, 1918, xii, 397-447.
[15] Hall: Jour. Am. Vet. Med. Assn., lv, N. S. viii, No. 4, July, 1919, pp. 416-423.
[16] Hall: Jour. Am. Vet. Med. Assn., April, 1919, lv, N. S., viii, No. 1, pp. 24-45.
[17] Hall and Foster: Jour. Am. Vet. Med. Assn., October, 1919, lvi, N. S., ix, No. 1, pp. 59-70.
[18] Hall, Wilson and Wigdor: Jour. Am. Vet. Med. Assn., August, 1919, lv, N. S., viii, No. 5, pp. 543-549.
[19] Hall: Jour. Am. Vet. Med. Assn., April, 1919, lv, N. S, viii, No. 1, pp. 24-45.
[20] Hall and Foster: Jour. Am. Med. Assn., 1917, lxviii, 1916.
[21] Hall: Jour. Am. Vet. Med. Assn., 1917, v, 342.
[22] Hall: Jour. Lab. and Clin. Med., 1917, ii, No. 5, pp. 347-353.
[23] Hall and Wigdor: Jour. Am. Vet. Med. Assn., November, 1919, lvi, N. S., ix, No. 2, pp. 169-177.
[24] Hall: Jour. Am. Vet. Med. Assn., April, 1919, lv, N. S., viii, No. 1.
[25] Hall and Wigdor: Jour. Am. Vet. Med. Assn., November, 1919, lvi, N. S., ix, No. 2, pp. 167-177.
[26] Hall and Wigdor: Jour. Am. Vet. Med. Assn., January, 1920, lvi, N. S. ix, No. 4, pp. 394-399.
[27] Hall and Hamilton: Jour. Pharmacol. and Exper. Therap., April, 1918, xi, (3), pp. 231-261.
[28] Salant and Nelson: Am. Jour. Physiol., March, 1915, xxxvi (4), pp. 444-463.
[29] Hall: Am. Jour. Vet. Med., August, 1918, xiii, No. 8, pp. 383-387.

THE LEUCOCYTIC PHASE OF HEMOCLASTIC SHOCK*

By Wm. S. Middleton, M.D., and Ethel Thewlis, Madison, Wis.

SINCE the comprehensive report of Widal, Abrami and Iancovesco,[1] the crise hémoclasique has been subjected to renewed scrutiny and criticism. The phenomena constituting this unusual reaction following the ingestion of a glass of milk were definitely held by the group working with Widal to result from a failure of the proteopexic function of the liver. In a word, clinically by a disturbance of the hepatic function in relation to its proper fixation of the split products of protein digestion, a syndrome develops which closely simulates the experimental results of peptone injections or those of a temporary Eck's fistula. To this explanation of a protein responsibility for the resultant leucocytic fall, arterial blood pressure fall and alterations in the coagulability and the refractive index of the blood, most authorities subscribe. Brown[2] has analyzed the clinical value of this procedure and concluded that the observations on the blood pressure and the numerical white cell counts constitute adequate criteria for practical purposes. Feinblatt,[3] in a study of eighty healthy men, firmly established the normal occurrence of a leucocytosis on the ingestion of a glass of milk. This confirmatory observation of the normal reaction lent particular significance to the occurrence of a leucocytic decline under similar conditions.

The crise hémoclasique is almost constantly observed in cases of catarrhal cholangitis; and the time-honored belief of an error in fat metabolism in this condition led to an investigation of the element of milk responsible for the depression of the leucocytic reaction in these cases. Widal and his coworkers[1] have reported absolutely negative results from tests in which the carbohydrate and the fatty elements of milk were exhibited alone; whereas casein in amounts corresponding to that contained in a glass of milk gave a prompt and severe reaction.†

Since elsewhere in this review of the subject these workers had urged the qualitative rather than the quantitative nature of the hemoclastic shock, it was deemed advisable to reduce the component elements of milk to smaller quantities retaining their relative proportions. Therefore, while the control of whole milk was kept at 200 gm., the quantity of the component elements was in each case divided by two, anticipating quantitative rather than qualitative alterations in the curves. Casein, butter-fat and lactose were used then in these observations in the amounts found in 100 gm. of whole milk. Lactal-

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†D'autre part, nous nous sommes assurés que, dans le lait, ce sont bien les substances azotées qui déterminent l'hémoclasie digestive; car chez des sujets qui présentaient après l'ingestion de ce liquide la crise hémoclasique caractéristique, ni l'absorption d'hydrates de carbone (50 gr. de lactose), ni celle de graisse (30 gr. de beurre essoré), n'ont produit la moindre altération de l'équilibre hématique. Par contre, après absorption de 8 gr. de caséine sèche, dose correspondent à celle contenue dans 200 gr. de lait, on obtient une réaction très intense et particulièrement précose.
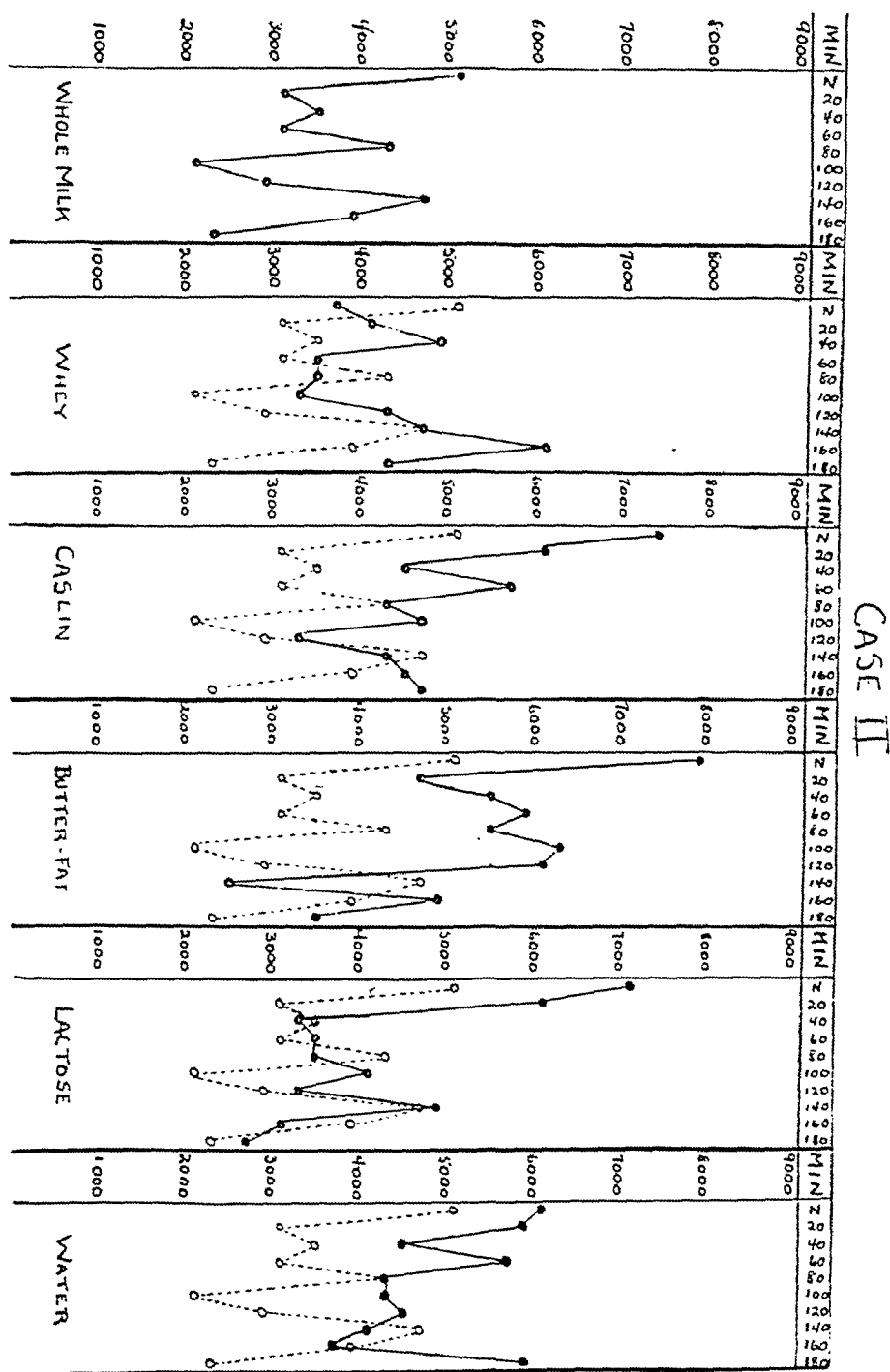
bumin, as such not being available, was given in the form of whey, realizing that lactose in amounts but little below that of whole milk was likewise administered in this procedure. After establishing the normal count in each case, at the same hour, on successive days the several tests were applied. The white cell counts were made every 20 minutes for three hours after the ingestion of the element to be tested. The curves in the three cases followed are appended. The whole milk curve is in each instance drawn in broken lines with the several curves of its component elements in solid lines for comparison.
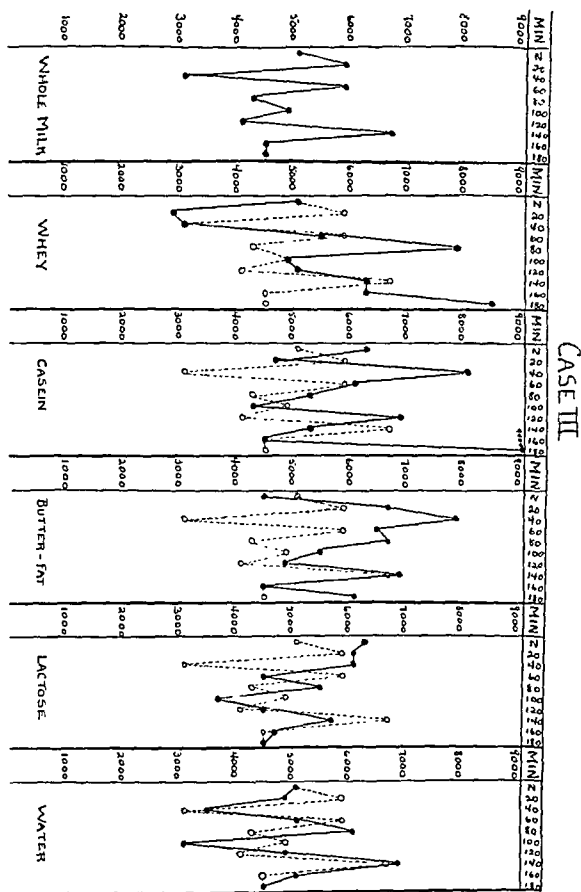
CASE I
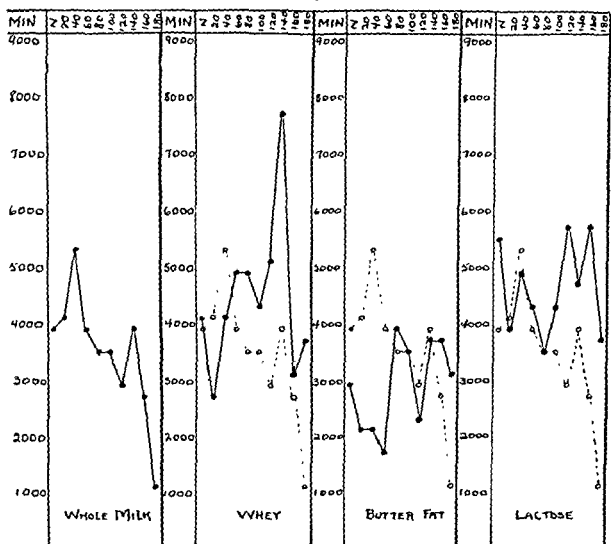
Analysis of these curves reveals certain obvious and illuminating facts. In none has an alimentary leucocytosis succeeded the ingestion of whole milk. The time of appearance of the leucocytic depression is inconstant as is also its degree. Three successive falls in the leucocytic curve may be traced in each instance. Contrary to the results of Widal,[1] casein showed a true shock response in only one of the two cases studied. In only the early phase of Case II does the curve of casein follow that of whole milk. The curves for whey, containing the other protein constituent of milk, are very bizarre, and incapable of reconciliation with their parent curves. Butter-fat on the other hand shows a resemblance to the whole milk curves in two of the three cases. Lactose significantly conforms most closely of all the studied elements of milk to the basic whole milk curve. This concurrence is best

exemplified in Cases II and III, wherein the form of the curves is very similar and only the time element varies. In Case I the degree of fluctuation in the lactose curve is greatly reduced as compared with whole milk, but the form of the curves coincides. The occurrence of a typical reaction on the inges-

tion of water in Case III casts doubt on the significance of the organic factor, protein or otherwise, in the causation of the blood changes. The degree of leucocytic fall with the reduced proportions of the elements of milk as compared with the basic curves of whole milk is surprising in many instances.

Clearly no conclusion can be drawn from such scanty data. However, the indication is clear that a protein element is not essential for the production of hemoclastic shock in cases of catarrhal cholangitis. The whole in-

teresting subject of hemoclastic shock should be critically analyzed before clinical deductions can be accurately drawn.

REFERENCES

[1]Widal, F., Abrami, P., and Iancovesco, N.: Presse méd., Dec. 11, 1920, No. 96, 893.
[2]Brown, J. R.: Ann. Clin. Med., September, 1922, i, No. 2, p. 89.
[3]Feinblatt, H. M.: Jour. Am. Med. Assn., March 3, 1923, lxxx, No. 9, p. 613.

THE DISTRIBUTION OF PHOSPHORUS COMPOUNDS IN THE BLOOD IN TUBERCULOSIS[*]

By K. Lucille McCluskey, Ph.D., Chicago, Ill.

I. INTRODUCTION

IT is a well established fact that phosphates are indispensable for the growth of the tubercle bacillus. The phosphorus is utilized undoubtedly for the formation of the nucleic acids of the nucleoproteins and the glycerophosphoric acids of the phosphatides, both of which are without question necessary for cell life.

In view of the important rôle of phosphorus in the metabolism of the tubercle bacillus it was considered worth while to study the distribution of phosphorus compounds in the cell and plasma and ascertain if possible, any tendencies toward progressive changes concomitant with the various stages of tuberculosis.

EXPERIMENTAL

Experimental data were obtained on the corpuscle-plasma ratio, the total phosphorus, the lecithin, the acid soluble, and the inorganic phosphorus of both whole blood and plasma. From the above, the cell content was calculated.

The subjects were male patients from the Municipal Tuberculosis Sanitarium of Chicago. The blood was drawn before breakfast and coagulation prevented by sodium oxalate. The phosphorus determinations were made according to the author's[1] method using trichloracetic acid as the protein precipitant, and the Sundstroem-Bloor[2] technic was used for the hematocrite readings.

The classification of the subjects is the same as that used in an earlier work[3] and is based both upon quantity and type of involvement. The diagrammatic scheme shows graphically the various courses over which the disease may travel. (See Chart I.)

Group VI is considered to be rather transitory and therefore difficult to classify. The early stages of the disease would *a priori* be of the neutral type (i.e., neither the destructive exudative type nor the proliferative healing type, but between these two extremes), with a moderate amount of involvement. This group is designated as III on the chart and represents a condition definitely recognized as tuberculous and first removed from the normal one in this scheme of classification. From Group III the disease may travel over one of three courses, viz.: III to IV; III to II to V; and III to II to I, depending upon the type and quantity of the involvement.

The results of the analyses expressed in mg. of orthophosphoric acid per 100 c.c. of whole blood, plasma, and cells (calculated) are given in Table I. A cytologic study is reported in Table II.

<p style="text-align:center">DISCUSSION</p>

The variations within the groups are no greater than in normal subjects, so that the averages of these various groups are assumed to be representative of the particular condition. All comparative studies are therefore based upon
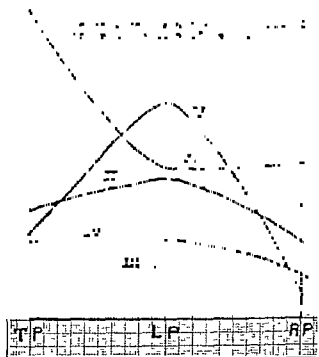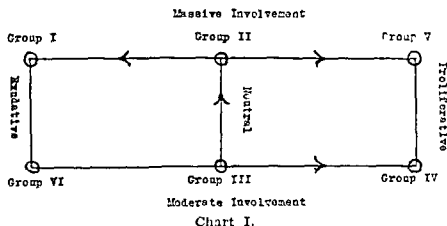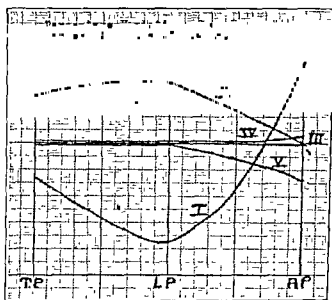

Chart I.

Chart II.

Chart III.

these averages. It is to be regretted that more subjects were not available for Group V, but very few patients of this class remain in a sanitarium.

In order to show changes in the distribution of phosphorus compounds as the disease passes through the various stages, it was thought best to use Group III as the basis for such comparison, since all the other groups have emanated from this particular one. Table III shows the percentage deviations from Group III as calculated from Table I. These percentage differences are graphed in Charts II, III and IV

It is to be borne in mind that these comparisons are based upon equal volumes of corpuscles, plasma, and whole blood (100 c.c.). Equal volumes

of cells do not necessarily contain the same number of cells. The number is a function of the size of the individual cell. Thus a study of changes within the cell should be based upon the content of the individual cell rather than upon unit volume.

That the average cell volume of Group III is about normal is evident if five million red cells, and a corpuscle volume of 45 per cent be taken as the average for the normal male, since the latter figures would give an average cell volume of .90 x 10 – 10 c.c.

From the red cell count, the corpuscle volume, and the phosphorus content per 100 c.c. of cells, the concentrations of the various phosphorus compounds in the cell were calculated and are given in Table IV, together with percentage changes in the size of the cell, in the corpuscle ratio and the phosphorus quotient. The data in the last three columns of the table are expressed in percentage variations from Group III.

TABLE I

PHOSPHORIC ACID CONTENT OF BLOOD OF TUBERCULOUS PATIENTS MG. OF $H_3 PO_4$ PER 100 C.C.

INITIALS AND CASE NO.	SEX	DATE	CLASSIFICATION GROUP	CORPUSCLE PLASMA RATIO COR.	CORPUSCLE PLASMA RATIO PL.	TOTAL PHOSPHORUS WHOLE BLOOD	TOTAL PHOSPHORUS PLASMA	TOTAL PHOSPHORUS CELLS (CALC.)	LECITHIN PHOSPHORUS WHOLE BLOOD	LECITHIN PHOSPHORUS PLASMA	LECITHIN PHOSPHORUS CELLS (CALC.)
M. L. (17973)	M	10-29-23	I	40.4	59.6	122.3	29.9	258.6	33.8	18.1	56.9
F. W. (16891)	M	12-21-23	I	32.0	68.0	116.6	35.6	288.7	33.5	22.2	57.5
M. L. (18253)	M	2-12-24	I	33.6	66.4	146.5	35.6	365.7	31.1	21.2	67.5
A. O. (5654)	M	2-12-24	I	37.1	62.9	125.0	35.0	272.2	36.7	23.9	58.4
C. M. (18454)	M	2-18-24	I	40.0	60.0	149.6	33.5	323.7	33.0	19.1	53.7
A. M. (18453)	M	2-18-24	I	34.6	65.4	140.4	29.0	353.1	28.3	14.7	54.3
AVERAGE GROUP I				36.2	63.7	133.6	31.1	310.3	32.7	19.8	58.0
J. S. (14581)	M	11-12-23	II	47.6	52.4	132.5	39.9	234.4	40.9	29.9	53.5
A. J. (16275)	M	11-12-23	II	47.0	53.0	129.3	33.6	237.2	38.9	23.9	55.9
J. S. (18036)	M	1-21-24	II	44.7	55.3	135.3	43.6	248.7	41.5	28.5	57.7
P. T. (18100)	M	1-21-24	II	42.6	57.4	137.3	33.3	277.4	34.9	18.7	56.8
J. O. (18147)	M	2-21-24	II	36.2	63.8	120.3	33.2	273.7	36.6	22.7	61.0
A. S. (14579)	M	10-29-23 .	II	41.4	58.6	131.1	35.6	266.4	36.3	20.4	58.6
AVERAGE GROUP II				43.2	56.7	130.9	36.5	256.3	38.2	24.0	57.2
J. O. (17977)	M	11- 8-23	III	38.6	61.4	134.4	36.8	289.6	39.2	25.0	61.9
A. H. (18001)	M	11- 8-23	III	43.4	56.6	117.3	31.9	220.2	32.4	21.4	46.7
O. J. (17464)	M	11-22-23	III	44.8	55.2	118.6	37.0	221.4	34.2	25.2	45.3
F. F. (17924)	M	11-22-23	III	45.8	54.2	127.5	42.6	228.1	35.7	28.7	44.1
T. M. (17475)	M	2-21-24	III	44.5	55.5	111.6	30.5	212.8	35.3	20.6	53.7
G. N. (18202)	M	2-25-24	III	42.1	57.9	118.5	30.1	240.1	33.9	17.4	56.7
F. C. (17592)	M	2-25-24	III	40.8	59.2	124.1	32.8	256.6	34.0	18.8	56.1
AVERAGE GROUP III				42.9	57.1	121.7	34.5	239.7	34.9	22.4	52.0
W. F. (16769)	M	10-24-23	IV	46.3	53.7	131.1	35.4	241.6	37.3	25.2	51.4
W. C. (17986)	M	10-24-23	IV	43.0	57.0	119.2	30.9	236.2	33.7	17.8	54.8
J. G. (18404)	M	2-25-24	IV	38.8	61.2	117.9	41.5	238.4	35.6	27.1	48.9
M. L. (18037)	M	2-25-24	IV	42.4	57.6	117.1	34.5	229.4	34.0	22.4	49.7
J. M. (17380)	M	11-28-23	IV	44.3	55.7	119.0	32.1	228.4	37.9	22.8	56.8
J. C. (18029)	M	1-21-24	IV	39.2	60.8	139.1	32.1	305.1	35.0	18.1	61.2
AVERAGE GROUP IV				42.3	57.7	123.9	34.4	246.6	35.6	22.2	53.8
J. W. (4130)	M	12-17-23	V	44.2	55.8	118.6	34.0	225.5	39.9	21.2	63.5
E. K. (10831)	M	12-17-2?	V	41.0	59.0	132.0	34.3	272.6	42.2	20.4	68.7
M. J. (13634)	M	11-22-?'	V	42.9	57.1	125.5	35.2	245.6	37.9	26.1	53.3
AVERAGE GROUP V				42.7	57.3	125.3	34.5	247.9	40.0	22.5	61.8

Contrasting Chart II with Chart V, it is evident that a rather erroneous impression of the actual conditions within the cell is obtained if such comparisons are based upon unit volume of cells instead of the cell itself. For this reason a comparison made upon the individual cell is considered preferable since it is the actual condition within the cell itself that is most to be desired.

It would appear from Chart II that the total phosphorus of the cell was increased over Group III in all groups, whereas it is actually less or equal in concentration with the exception of Group I. The same is true of lecithin and similar relations with the acid soluble phosphorus fraction exist.

A summary of Charts III and V indicate that as the disease passes from the moderate involvement neutral type (Group III) to the moderate involvement proliferative type (Group IV) the only change from Group III is a decrease in acid soluble phosphorus in the cell and a slight increase in the

TABLE I—CONT'D

PHOSPHORIC ACID CONTENT OF BLOOD OF TUBERCULOUS PATIENTS MG. OF $H_3 PO_4$ PER 100 C.C.

ACID SOLUBLE PHOSPHORUS			LECITHIN PLUS ACID SOLUBLE PHOSPHORUS			TP—(ASP PLUS LP)			INORGANIC PHOSPHORUS		
WHOLE BLOOD	PLASMA	CELLS (CALC.)	WHOLE BLOOD	PLASMA	CELLS	WHOLE BLOOD	PLASMA	CELLS	WHOLE BLOOD	PLASMA	CELLS (CALC.)
89.1	11.3	204.4	129.9	29.0	261.3	−0.6	+0.9	−2.7	17.8	10.6	28.9
76.2	13.6	209.3	109.7	35.8	266.8	+6.8	−0.2	+21.9	13.2	13.0	13.7
102.0	14.1	218.5	133.1	35.3	346.0	+13.4	+0.3	+19.7	19.5	12.5	33.3
86.8	14.5	209.1	123.5	38.4	267.5	+1.5	−0.1	+4.7	19.3	13.7	31.5
106.9	13.2	247.5	139.9	32.3	301.2	+9.7	+1.2	+22.5	10.2	11.3	8.5
102.6	14.1	271.5	130.9	28.8	325.8	+9.5	+0.2	+27.3	11.4	14.1	2.9
92.9	13.4	226.7	126.6	33.2	294.7	+6.7	+0.4	+15.5	15.2	12.5	19.8
92.7	12.4	181.1	133.6	41.9	234.6	−1.1	−2.0	−0.2	13.5	12.0	1.3
92.1	11.7	182.7	131.0	35.6	238.6	−1.7	−2.0	−1.4	14.9	11.5	18.7
92.9	13.1	191.7	134.4	41.6	249.4	+0.9	+2.0	−0.7	13.4	11.7	15.4
100.7	14.0	217.6	135.6	32.7	274.4	+1.7	+0.6	+3.0	14.4	11.5	18.3
84.3	10.8	213.8	120.9	33.5	213.8	−0.6	−0.3	−1.1	8.8	10.9	5.2
94.4	11.3	212.0	130.7	31.7	271.1	+0.4	+3.9	−4.2	20.3	11.0	33.5
92.8	12.2	199.8	131.0	36.1	246.9	+0.0	+0.3	−0.7	14.2	11.4	15.4
95.6	12.2	222.8	134.8	37.2	284.7	−0.4	−0.4	+4.9	12.5	11.0	15.0
87.9	12.1	186.8	120.3	33.5	233.5	−3.0	−1.6	−4.3		11.1	
86.7	12.3	221.4	120.9	37.5	221.4	−2.3	−0.5	+0.0			
91.2	12.0	184.9	126.9	40.7	229.0	+0.6	+1.9	−0.9	17.7	11.0	15.2
76.0	10.8	158.6	111.3	30.3	212.3	+0.3	+0.2	+0.5	7.6	9.9	4.9
85.3	13.1	184.5	119.2	30.5	184.5	−0.7	−0.4	−1.1	17.6	14.6	21.8
88.9	13.4	197.5	122.9	32.9	253.6	+1.2	−0.1	+3.0	17.9	14.5	22.7
87.3	12.2	193.8	122.3	34.6	231.3	−0.6	−0.1	+0.3	14.6	12.0	15.9
93.7	11.6	189.9	131.0	36.8	240.3	−0.1	−1.4	+1.3	14.2	9.0	20.3
86.2	13.6	182.3	119.9	31.4	237.1	−0.7	−0.5	−0.9	16.5	11.0	23.7
81.2	13.4	188.1	116.8	40.5	237.0	+1.1	+1.0	+1.4	17.8	11.8	27.3
81.7	12.4	175.9	115.7	34.8	225.6	+1.4	−0.3	+3.8	16.5	12.6	21.6
79.4	9.9	166.8	117.3	32.7	223.6	+1.7	−0.6	+4.8	13.1	9.9	21.2
104.5	14.8	243.6	139.5	32.9	304.8	−0.4	−0.8	+0.3	15.8	12.4	21.1
87.8	12.6	190.9	123.3	34.8	244.7	+0.5	−0.4	+1.8	15.6	11.1	22.5
80.6	11.6	167.8	120.5	32.8	231.3	−1.9	+1.2	−5.8	12.8	11.8	14.0
90.0	12.4	201.7	132.2	32.8	270.4	−0.2	+1.5	+2.2	15.7	11.9	21.2
86.7	11.1	187.6	124.6	37.2	240.9	+0.9	−2.0	+4.7	18.3	11.0	27.9
85.8	11.7	185.7	125.7	34.3	247.5	−0.4	+0.2	+0.3	15.6	11.5	21.0

TABLE II

INITIALS AND CASE NO.	SEX	AGE	GROUP	RED CELL COUNT	VOLUME PER CENT OF CELLS	CELL VOLUME × 10-10 (C.C.)	LEUCO-CYTES	REMARKS
M. L. (17973)	M	38	I	4.73 MM	40.4	0.85	7.3 M	
F. W. (16891)	M	30	I	4.65	32.0	0.68	13.9	Patient died 10 days later
M. L. (18253)	M	34	I		33.6			Patient died 24 hours later
A. O. (5654)	M	33	I	4.54	37.1	0.81	11.0	
C. M. (18454)	M	31	I	4.93	40.0	0.81	8.6	Patient died 5 days later
A. M. (18453)	M	30	I		34.6			Patient died 24 hours later
AVERAGE GROUP I		32		4.71	36.2	0.79	7.7	
J. S. (14581)	M	49	II	5.85	47.6	0.81	9.3	
A. J. (16275)	M	21	II	4.94 5.46	47.0	0.95	8.9	Patient died 4 months later
J. S. (18036)	M	21	II		44.7	0.82	9.8	
P. T. (18100)	M	31	II	4.84	42.6	0.88	14.5	
J. O. (18147)	M	37	II	4.98	36.2	0.72	9.9	
A. S. (14579)	M	36	II	4.97	41.4	0.83	8.8	
AVERAGE GROUP II		30		5.17	43.2	0.83	10.2	
J. O. (17977)	M	27	III	4.82	38.6	0.80	10.6	
A. H. (18001)	M	27	III	4.47	43.4	0.97	10.6	
O. J. (17464)	M	26	III	5.13	44.8	0.87	7.1	
F. F. (17924)	M	25	III	4.23	45.8	1.08	9.6	
T. M. (17475)	M	38	III	4.60	44.5	0.97	8.2	
G. N. (18202)	M	19	III	4.81	42.1	0.87	8.5	
F. C. (17592)	M	27	III	4.76	40.8	0.85	14.0	
AVERAGE GROUP III		27		4.69	42.9	0.91	9.8	
W. F. (16769)	M	27	IV	4.97	46.3	0.93	8.9	
W. C. (17986)	M	34	IV	5.00	43.0	0.86	7.8	
J. G. (18404)	M	68	IV	4.84	38.8	0.80	7.8	
M. L. (18037)	M	31	IV	4.76	42.4	0.89	5.6	
J. M. (17380)	M	38	IV	4.33	44.3	1.02	7.5	
J. C. (18029)	M	38	IV	4.98	39.2	0.79	10.5	
AVERAGE GROUP IV		39		4.81	42.3	0.88	8.0	
J. W. (4130)	M	53	V	5.03	44.2	0 83	9.5	
E. K. (10831)	M	40	V	4.60	41.0	0.89	11.6	
M. J. (13634)	M	39	V	4.85	42.9	0.88	10.4	
AVERAGE GROUP V		44		4.83	42.7	0.86	10.5	

TABLE III

PERCENTAGE VARIATIONS FROM GROUP III PER 100 C.C.

GROUP	TOTAL PHOSPHATE			LECITHIN PHOSPHATE			ACID SOLUBLE PHOSPHATE		
	CELLS	PLASMA	WHOLE BLOOD	CELLS	PLASMA	WHOLE BLOOD	CELLS	PLASMA	WHOLE BLOOD
I	+29.4	−4.0	+9.7	+11.5	−11.6	− 6.3	+11 8	+9.8	+6.4
II	+ 6.9	+5.7	+7.5	+10.0	+ 7.0	+ 9.4	+ 3 0	0.0	+6.3
IV	+ 2.8	0.0	+1.8	+ 3.0	0.0	+ 2.0	− 1.0	+1.0	0.0
V	+ 3.4	0.0	+2.9	+18.8	0.0	+14.5	− 3.0	−4.0	−2.0

plasma. Group II contains more lecithin in the plasma and about the same in the cell as Group III with a decrease in acid soluble phosphorus in the cell. Group V is characterized by a considerable increase in lecithin content in the cell, with a decrease in acid soluble phosphorus in both cell and plasma. It is to be noted that the lecithin is increased in the plasma in Group II. Since

Group II lies next to either extreme (Groups I and V) it is rather difficult to speculate on the cause of this increase. It might represent the passage of lecithin from the cell to the plasma as would be expected in going from Group II to Group I, or an accumulation of lecithin in the plasma preparatory to entering the cell in passing from Group II to Group V. Group I is quite the opposite of Group V with a low lecithin content in both the cell and the plasma, with a high increase in the acid soluble phosphorus in the plasma. The total phosphorus of the cell in Group I is high, though both the lecithin and the acid soluble phosphorus are depressed. This is explained by the presence of a phosphorus fraction in the cell which is not included in the phosphatides or acid soluble groups.

It is to be noted in Table I, Group I, that there is a difference between the total phosphorus and the sum of the lecithin and acid soluble fractions in the whole blood and the cells, but not in the plasma, indicating that this

Chart IV
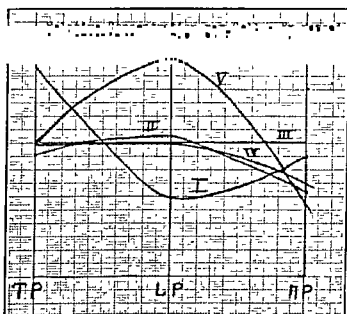
Chart V.

phosphorus fraction is confined to the cell. Within experimental error the sum of the lecithin and acid soluble phosphosus is equal to the total phosphorus in normal individuals and this is also true in Groups II, III, IV and V. The significance of this phosphorus residue clearly indicates that it is a fraction precipitated by the ether-alcohol mixture and the trichloracetic acid. Found coincident with the terminal stage of the disease is indicative that it may represent a nucleoprotein or an abnormal decomposition product of the latter or possibly a product of metabolism of the tubercle bacillus found either free or bound to some phosphorus compound in the cell.

From Table IV it is to be observed that the actual cell volume is decreased in all the groups, particularly Group I. A possible explanation is that the water is drawn out of the cell by either an increase in the osmotic pressure of the plasma occasioned by an increase in plasma solids or a disturbance in the water balance equilibrium between the blood and the tissues.

The phosphorus quotient is obtained by multiplying the ratio between the total phosphorus in 100 c.c. of cells and the number of red cells in the same

volume by 10^{10}. In the same column, in parenthesis, are found the percentage variations from Group III. The phosphorus quotients of Groups III, IV, and V are identical while that of Group II is very slightly increased and Group I much more so.

It is not assumed that the white cells are phosphorus free, but the leucocytes are relatively few in comparison to the number of erythrocytes.

The inorganic phosphorus in the plasma is higher than previous reports on normal subjects. It apears to be practically equal to the acid soluble phosphoric acid fraction of the plasma. Denis[4] has recently reported that there is no inorganic phosphoric acid in the cell in the normal subject, while previously reports an average of 18.8 mg. per 100 c.c. of cells. Since as not possible to use the technic suggested by Denis in this study, not much emphasis can be placed upon the values obtained for inorganic phosphate in whole blood or cells; still it may represent a comparable fraction which if not present as inorganic phosphate is one that may readily be converted into the same.

Table VI shows a contrast between Group III and the average figures for normal men given by Bloor.[6] The most notable difference lies in the decrease of lecithin in the cell with an increase in the acid soluble phosphorus fraction in both the cell and the plasma. Even in the early stages of the disease there appears a tendency toward a decrease in lecithin and an increase in acid soluble phosphorus.

Charts VI and VII indicate the manner in which the lecithin and the acid soluble phosphates fluctuate from normal as the disease passes through Groups III to II to I, and III to II to V respectively. These percentage differences

TABLE IV

MG. OF H_3PO_4 PER INDIVIDUAL CELL

GROUP	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS	PERCENTAGE VARIATION OF CELL VOLUME FROM GROUP III	PERCENTAGE VARIATION OF CORPUSCLE RATIO FROM GROUP III	PROSPHORUS QUOTIENT
I	2.38×10^{-10}	$.446 \times 10^{-10}$	1.74×10^{-10}	-13.1%	-15.6	2.38 (+8.6%)
II	2.15×10^{-10}	$.480 \times 10^{-10}$	1.67×10^{-10}	-8.7%	$+0.7$	2.15 (−1.8%)
III	2.19×10^{-10}	$.477 \times 10^{-10}$	1.77×10^{-10}			2.19
IV	2.18×10^{-10}	$.476 \times 10^{-10}$	1.68×10^{-10}	-3.4%	-1.0	2.18 (−0.4%)
V	2.19×10^{-10}	$.524 \times 10^{-10}$	1.64×10^{-10}	-5.4%	-0.5	2.19 (0.0%)

TABLE V

SHOWING PERCENTAGE CHANGES FROM GROUP III IN THE INDIVIDUAL CELL

GROUP	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS
I	+8.6%	−6.5%	−1.7%
II	−1.8%	+0.6%	−5.6%
IV	−0.4%	−0.2%	−5.0%
V	0.0%	+9.8%	−7.3%

are obtained by comparison of the results in Tables V and IV (plasma) with the corresponding phosphorus content of the cell and unit volume of plasma of normal men calculated upon the basis of Bloor's figures assuming that the average volume of the normal male red cell is 90×10^{-10} c.c. and that the number of erythrocytes per 100 c.c. is 111×10^{9} cells. From Charts VI and
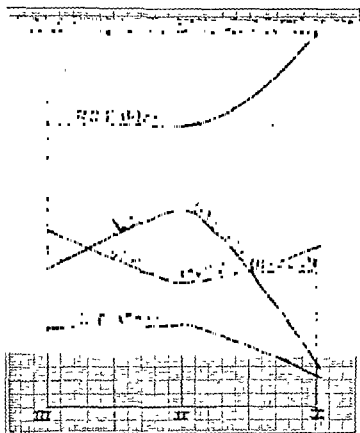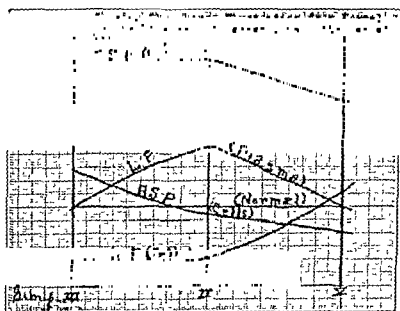

Chart VI.

Chart VII.

VII, it is to be noted that in general the greatest variation occurs in the acid soluble phosphorus of the plasma and the lecithin content of the cell. As one fraction increases, the other simultaneously decreases and vice versa. There is a similar relationship existing between the lecithin content of the plasma and the acid soluble phosphorus of the cell, but these differences are not so exaggerated.

TABLE VI

MG. OF H_3PO_4 PER 100 C.C.

GROUP	CELLS			PLASMA		
	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS	TOTAL PHOSPHORUS	LECITHIN PHOSPHORUS	ACID SOLUBLE PHOSPHORUS
Bloor's Group Average	248.0	57.0	188.0	32.0	22.1	10.4
Group III Average	239.7	52.0	193.8	34.5	22.4	12.2

SUMMARY

1. There is a disturbance in phosphorus metabolism in tuberculosis.

2. A decrease in lecithin content of both the cell and the plasma with a simultaneous increase in acid soluble phosphorus represents grave prognosis while the opposite indicates a favorable condition.

3. Since the decrease in lecithin is accompanied by an increase in acid soluble phosphorus it would appear that the latter fraction may be utilized for the synthesis of lecithin. In the advanced stages of tuberculosis there may be a destruction of lecithin or an inability to synthesize it, occasioned by a lack within the body of fatty acids, the choline residue, glycerol or perhaps specific enzymes.

4. The size of the red cell is decreased in tuberculosis, except in the first stages, and the volume per cent of cells is decidedly decreased in the far advanced exudative type.

5. From Table II it is interesting to note the average age of patients in the different groups. It is to be observed that the youngest patients are found in Group III, indicating what has long been known that tuberculosis is a disease of early adult life. That the period of healing is proportional to the amount of involvement is observed by comparison of the two proliferative groups. If the disease is not arrested it passes in a few years to the terminal stage.

6. The sum of the lecithin and acid soluble fractions is equal to the total phosphorus except in Group I, terminal stage, in which the total phosphorus is greater than the sum of these two fractions. This additional phosphorus fraction is confined to the cell and represents a group not soluble in ether-alcohol or trichloracetic acid.

BIBLIOGRAPHY

[1]McCluskey, K. L.: JOUR. LAB. AND CLIN. MED., 1925. (In press.)
[2]Sundstroem and Bloor: Jour. Biol. Chem., 1920, xi, 157.
[3]Sweany, Weathers and McCluskey: Amer. Rev. Tuberc., 1924, viii, 405.
[4]Denis, W.: Jour. Biol. Chem., 1923.
[5]Bloor, W. R.: Jour. Biol. Chem., 1918, xxxvi, 33.
[6]Bloor, W. R.: Jour. Biol. Chem., 1918, xxxvi, 49.

A NOTE ON THE DESTRUCTION OF COMPLEMENT IN HUMAN SERUM BY HEATING AT 55° C. FOR FIFTEEN MINUTES*

By Gordon Ernest Davis,† M.S., Mobile, Ala.

IN spite of the work done by Kolmer and others on the destruction of complement in human serum by heating at 55° C. for fifteen minutes instead of from twenty to thirty minutes as formerly used, there is still an occasional echo of doubt as to the efficiency of the shorter period of time. To determine this point for myself, I tested the serum from 500 routine blood specimens as they came to the laboratory from seven counties over a period of nearly two months. The Wassermann tests are begun on Tuesday and Friday of each week. All bloods are placed in the ice box as received and the serum removed just before testing.

I felt that if after inactivation the complement remained sufficient to even weaken a small part of the regulation dose of cells, then a longer period should be used.

A two-tube series was set up sufficient for the sera of the day. In the back tubes I placed 0.1 c.c. of raw serum and in the front tubes the same amount of inactivated (fifteen minutes at 55° C.) serum. One-tenth c.c. (one-fifth of the regulation dose) of the red cell suspension made up for the day was then added to all tubes and the total made up to 3 c.c. with physiologic saline. The back tubes thus acted as controls on the front tubes. All tubes were incubated at 38° C. for one hour.

Of the 500 sera, twenty-six failed to hemolyze before heating, i.e., these sera contained insufficient complement or native anti-sheep hemolysin, or both to produce hemolysis in the suspension of cells used. Of the 474 sera which gave complete hemolysis before inactivation, not one showed any detectable degree of hemolysis after inactivation. Five hundred sera is not a very long series but I feel that the results are fairly conclusive of the efficiency of the short period of inactivation.

FIBROID GUMMA*

(Juxta-Articular Nodules of Lutz-Jeanselme)

By Sigmund S. Greenbaum, M.D., Philadelphia

THE syphilitic gumma in the form of moderately firm asymmetrical nodules, running a characteristic course, as a rule towards ulceration in a fairly definite period of time, is well known; but that syphilis may be the primary etiologic factor in the production of very hard, often symmetrically located, intracutaneous and subcutaneous fibrotic nodules, almost pathognomonic when their clinical picture presents a certain topography, is practically unknown. Although the appearance of these nodules generally causes no symptomatic disturbances, they are important to recognize from a diagnostic point of view. There are few instances of this manifestation of syphilis, recorded as such, in the literature, nor have I been able to find mention of the condition in most of the current textbooks on syphilis.

Because of the recent observation of a typical example in a syphilitic, who had a primary lesion eleven years previously and who presented symmetrically on the elbows, on the right knee and on the left palm, very hard hypodermic and dermo-hypodermic nodules of six to eight years duration and because of their apparent rarity and the lack of uniformity regarding their etiologic significance, a clinical review of this combined syphilitic fibro-gummatous lesion and its relation to the fibromata and the juxta-articular nodules, so common in the orient and tropical countries, appears desirable. The history of the patient forming the basis for this is as follows:

L. A., white male, salesman, aged forty-five, was referred by Alfred Cowan, March 13, 1924, with the diagnosis of chronic syphilis, a complement-fixation test having been performed because of recurrent attacks of corneal ulcer.

The patient was American born and had been married eighteen years. There was one child aged fifteen years; his wife had had one miscarriage preceding the birth of this boy and had one miscarriage after his birth but before the husband had contracted syphilis. The patient stated that he did not recall having any serious illness during his life except for the chancre which he had long since forgotten. The fibroid gummata caused him no trouble and were of no interest to him whatever. This is possibly the reason that so few instances of this condition have been noted in the literature. The first lesion to appear was on the hypothenar eminence of the left hand in the form of a hard nodule—the patient stated that it felt like a small marble right in the skin of the palm. The others appeared in the following order: left elbow, right elbow, thenar eminence left hand and lastly on the right knee. They had, at no time, been painful or tender. Those over the large joints had been soft and ''spongy'' at first, readily movable under the skin and had later become very firm. There was no history of a secondary luetic eruption and the patient stated that he had never felt better in his life.

Examination.—The patient, an exceedingly well-preserved and well-nourished man of medium height, weighed 162 pounds and was apparently in the best of health and spirits.

Except for a moderately marked granular pharyngitis, the nose and throat examination was negative. The heart was apparently normal except for a slightly accentuated aortic second sound. The lungs and abdominal viscera were apparently normal. The systolic blood pressure was 130. The urinalysis was negative.

On both forearms, over the olecranon processes, about one to two inches above the elbow tips, were very hard nontender subcutaneous globular nodules. Although very freely movable, they were attached to the overlying skin to a definite degree by their superior poles. Although symmetrical as to position, they were not of the same size. The lesion on the left elbow was much larger and about the size of a moderate sized hen's egg. Of all the lesions, this was the most easily movable under the skin and was distinctly globular. The lesion on the left elbow was about the size of a pigeon's egg and had a very small hard additional nodule attached to its deeper surface laterally. The nodule on the right knee had the same characteristics as those on the elbows except that it was much smaller. The

Fig. 1.—Fibroid gummata over olecranon processes. (Juxta-articular nodules of Lutz-Jeanselme.)

lesions on the palms were somewhat different from the others. They were at the same time both intra- and hypodermic. They were about the size of navy beans and the skin over them was hyperkeratotic. None of the nodules bore any relation to the bursae.

The Wassermann test was four plus with all antigens and with several technics. No histologic examination was made as the patient refused to permit a biopsy.

Treatment and Results.—Within one week after the first injection intravenously of a small dose of neoarsphenamine (.30 gm.), the patient noted a perceptible diminution in size of the smaller lesions on the palms. By the fourth injection, there was distinct shrinkage in the size of all the lesions while the palmar lesions had lost all sensation of being nodular.

All that remained of the palmar lesions were hyperkeratotic spots. At the end of the first series of arsenical injections (12.6 gm.) the palmar lesions had completely disappeared and the juxta-articular nodules had regressed fully 75 per cent. There was almost complete disappearance of all lesions at the end of the second series of arsenical injections which had been preceded by 30 intramuscular injections of bismuth.

This case is a typical example of a condition observed by Lutz in 1891 and later by Jeanselme whose clinical description is classical and who gave it the term juxta-articular nodules.[1] At that time, Jeanselme stated that the condition was neither syphilitic nor tuberculous and a practical application of the Bordet-Gengou phenomenon had not as yet been discovered. More recently, in England and America, several cases, in which the clinical picture was similar to that of juxta-articular nodules, have been described by Weber,[2] Goodman and Young,[3] Goodman[4, 5] and Fox,[6] under the term subcutaneous fibroid syphilomata. That the two conditions are the same is admitted by Jeanselme in a recent review of the subject appearing during the preparation of this report,[7] although he does not as yet admit a common primary etiologic factor.

Juxta-articular nodules, as observed by Jeanselme among the Indo-Chinese, are rounded or poly-lobulated, hard, indolent, lesions of varied size, which, as their name indicates, tend to develop or specially locate near joints.

In this special location, they tend to assume a remarkable symmetry and on this, chiefly, rests their diagnostic value. The nodules are at first subcutaneous and freely movable but later, often become attached to the overlying skin. After reaching a certain point in their development, they generally persist as such, although very exceptional instances have been recorded of softening, evacuation, ulceration and cicatrization. Since Jeanselme's original observation of this condition, it has been noted over widely distributed areas in the orient, Africa and south America, with apparently sporadic cases here and there in North America, England and the Continent. The majority of observers have been able to definitely ascertain that the common carriers of juxta-articular nodules are individuals with an antecedent history of syphilis or yaws and that the complement-fixation test for syphilis is frequently positive. In one instance, spirochetes were found in silver-nitrate impregnated sections of a nodule.[8] In the hands of most investigators, excellent therapeutic results have followed antisyphilitic, particularly arsenobenzene, therapy. It appears therefore that sufficient evidence has accumulated to definitely establish at least one of the factors in the production of juxta-articular nodules.

Connected with the etiology of juxta-articular nodules is the question of the etiology of fibromata in general. It is well known that the history of fibromata (the term in many textbooks includes the so-called true and pseudo-fibromata) is not always uniform and that the histologic appearance frequently depends upon the time, as related to the stage of development, when the section is made. A survey of the pathologic descriptions of fibromata shows that there is, at times, a fairly marked similarity between the description of some of them and the description of the histologic appearance of juxta-

articular nodules. As a matter of fact, the clinical appearance is that of multiple fibromata but it must be remembered that this is not the fact pathologically. They are not true fibromata. Nevertheless, it is of interest to note that Stelwagen, quoting Hashimoto, states that fibromata (?) are extremely common in the eastern countries whereas they are comparatively uncommon in this country and in Europe. Juxta-articular nodules usually occur at pressure points (ankles, knees, elbows). The positions assumed by the peoples in the eastern countries, when praying, resting, etc., as well as the tendency of the dark-skinned and the exceptional white-skinned individual to fibrotic hypertrophies explains the localizations and the pathologic composition of fibroid gummata. Since juxta-articular nodules are of extreme frequence in the areas similarly noted by Hashimoto for fibromata, the inference that many of these latter are really juxta-articular nodules or fibroid gummata is justifiable. They are really another illustration of the effect of trauma on the development of syphilitic lesions plus the fibrotic tendency of the individual.

There is a growing desire among dermatologists to avoid the term tubercle in describing a certain elementary skin lesion, and to substitute for it, wherever possible, the more descriptive term lupoma, leproma, etc. Just as the term lupoma or leproma replaces the obsolete term lupus tubercle, leprous tubercle, the term syphiloma should be restricted to the nonresolutive syphilitic tubercle in which there is dermic destruction with scar formation (cutaneous fibrosis) as the end-result

The term fibroid gumma clearly describes the condition.

REFERENCES

[1]Jeanselme and Rist: Précis de pathologie exotique, Paris, 1909, Masson and Cie.
[2]Weber, F. P.: Brit. Jour. Derm., June, 1920, xxxii, 173.
[3]Goodman, H. and Young, W. J.: Am. Jour. Med. Sc., February, 1921, clix, 231.
[4]Goodman, H.: Brit. Jour. Derm., October, 1921, xxxiii, 335.
[5]Goodman, H.: Am. Jour. Syph., 1922, vi, 687.
[6]Fox, H.: Arch. of Derm. and Syph., February, 1922, v, 198.
[7]Jeanselme, E.: Paris med. Jour., March 15, 1924, xi, 242.
[8]Van dijke and Oudenthal, A. J.: quoted by Jeanselme, loc. cit.

URIC ACID LEVELS IN THE BLOOD OF MAN AND ANIMALS*

By A. T. Weathers, B.S., and H. C. Sweany, M.D., Chicago

INTRODUCTION

THE value of a reliable uric acid method for the control of such a disease as nephritis is obvious, but until quite recently no such methods were available. Formerly from 1 to 3 milligrams per 100 c.c. was considered normal. but recent work has shown that the methods used to establish this record were faulty. The purpose of this work therefore is to ascertain if possible the most reliable and at the same time the most practical method, to discover any source of error hitherto unrecognized, and to establish various levels of concentration for animals and man.

URIC ACID METHODS

Prior to the introduction of the phosphotungstic acid reagent of Folin and Denis, uric acid was determined gravimetrically, a procedure which required 50 to 100 c.c. of blood, making analysis almost impossible for routine procedure. In 1913 Folin and Denis developed a colorimetric method by means of which it was possible to make an accurate determination on 20 c.c. of blood. In 1915, Benedict and Hitchcock modified the Folin and Denis method so that it was possible to make an analysis on 10 c.c. of blood. In 1919, Folin and Wu first described the use of tungstic acid wherein they were able to obtain a protein free filtrate on which all nonprotein constituents of the blood could be determined. They were able to make the uric acid analysis on sufficient of this filtrate, to represent 2 c.c. of blood. In 1922, Benedict[1] introduced a new arsenic-tungstic acid reagent with which he brought out a very accurate method for uric acid in the Folin-Wu[2] tungstic acid filtrate. In Benedict's new technic, the test was made on this filtrate representing 0.5 c.c. of blood.

The Folin-Wu method, using the equivalent of 2 c.c. of blood was epoch-making. The determination was made by precipitating the uric acid from 20 c.c. of Folin-Wu filtrate as the silver urate by means of silver lactate-lactic acid solution. The urate was thrown down in a centrifuge, separated from the supernatant liquid by decantation and then dissolved by a hydrochloric acid solution of sodium chloride. The chloride solution of the silver urate was transferred to a 25 c.c. graduated flask, made alkaline with sodium carbonate and sodium cyanide. A uric acid standard was prepared containing equal amounts of carbonate and cyanide as used in the blood analysis and a blue color developed by adding Folin and Denis reagent.[7] This method has been criticized by Rogers,[6] Benedict[1] and by Brown and Raiziss.[4]

H. Rogers has shown that the Folin-Wu method is subjected to a very serious source of error when silver urate is exposed to light. We also noted that this darkening of the precipitate gave low results and had always prevented it. Benedict points to the facts: that in the Folin-Wu method, the uric acid is precipitated from a blood filtrate where its actual concentration may be even less than one part in a million; that uric acid cannot be precipitated in pure solution of ten times the concentration existing in blood filtrates; that the color produced is too weak to make accurate comparisons with standards; that the sulphite standard was subject to slow deterioration; and that sulphites interfered with the development of blue color in the analysis. Brown and Raiziss state that the results from the Folin-Wu test are 6 to 10 per cent too low but those from the new Benedict technic are too high. We have not been able to agree with the authors on these points or to the effect of polyphenols and aminoacids on the color development in uric acid analysis with the Benedict or Folin and Denis reagent (See Tables IV and V).

In Benedict's new method, the test is made directly on 5 c.c. of Folin-Wu filtrate in a weak sodium cyanide solution. The color is developed by an arsenic-tungstic acid reagent, by heating three minutes in a boiling water-bath. The color so produced is about three times as strong as that developed in the Folin-Wu method and better colorimetric readings can be made thereby. In Table I, are shown the results of analyses using the new Benedict and the technic of Folin-Wu. In seventeen of twenty cases the Benedict gives higher results than the Folin-Wu. Following this in Table I, are shown the results of the new Benedict and Benedict's modification[1] of the Folin-Wu, uric acid method. In seventeen of eighteen analyses the Benedict technic gives higher results.

In Table II, results are given by the new Folin[3] and the new Benedict[1] methods, each method giving a fairly accurate per cent of recovery. Folin's supplement to "A System of Blood Analyses"[3] deals with the criticism of Pucher[10] and Benedict,[1] and outlines a new direct colorimetric method for uric acid similar to that of Benedict, using 5 c.c. of the Folin-Wu filtrate with the Folin and Denis reagent. This results in the development of a much stronger color than in any of the previous methods. Furthermore, by employing lithium salts instead of sodium, Folin has eliminated any chance of cloudy solutions that may arise from time to time in making analysis as outlined by Benedict.

EXPERIMENTAL

Comparisons between the new Folin and new Benedict technics were then carried out and at the same time a method, using the Folin technic with a mixed reagent of three parts of Folin and Denis to one part of the Benedict arsenic-tungstic acid reagent. The results of twenty-seven analyses are shown in Table III. The difference of the two methods is always very small and within the limits of experimental error, a fact not true with the old Folin-Wu when compared with Benedict's method. As the Benedict arsenic reagent is supposed to give an increase and the Folin and Denis reagent a decrease in

color in the presence of polyphenols, by adding one part of the former to three parts of the latter, a reagent was made that should not give an increase or decrease in color with interfering substances. However, this mixed reagent gave the same results as the other two with bloods of normal phenol and uric acid content, as shown in Table III.

Analyses were then made to determine the excess color development with reagents on the addition of polyphenols to the blood. Phloroglucin (1-3-5- trihydroxyphenol) was added in the ratio of 3 mg. to 100 c.c. of blood giving double the concentration of phenols found in normal bloods. The results of Table IV show that the effect of polyphenols on the uric acid determination is negligible when using either the Benedict or Folin and Denis reagent.

In Table V are results obtained by adding equal parts of tyrosine and aspartic acid equivalent to 7 mg. of amino-acids per 100 c.c. of blood. Even

TABLE I

NUMBER BLOODS TESTED	DIAGNOSIS	AVERAGE S. R. BENE-DICT'S TECHNIC	AVERAGE FOLIN-WU TECHNIC	DIFFERENCE BENEDICT'S AND FOLIN'S
2	Incipient TB.	4.77	3.86	0.91
10	Mod. advanced	4.14	3.06	1.02
4	Far advanced	3.14	3.13	0.01
		S. R. Benedict's technic	Benedict's Modification of Folin-Wu Technic	
1	Incipient	3.7	3.0	0.7
6	Mod. advanced	3.82	2.88	0.94
6	Far advanced	3.63	2.68	0.95
2	Nephritic	5.4	5.4	0.0
1	Uremic	4.7	5.8	–1.1
4	Normal	4.06	3.06	1.0

NOTE: Results in mg. per 100 c.c. of blood.

TABLE II

RECOVERY OF URIC ACID ADDED TO THE BLOOD BY BENEDICT'S NEW AND FOLIN'S NEW METHODS

CASE NO.	TECHNIC USED	URIC ACID IN BLOOD	URIC ACID ADDED MG. PER 100 C.C.	TOTAL URIC ACID RECOVERED	ADDED URIC ACID RECOVERED	PER CENT RECOVERED
16969	Benedict	3.3	3.0	6.3	3.0	100.00
	Folin	3.3	3.0	5.9	2.9	96.66
16982	Benedict	3.45	2.0	5.5	2.05	102.5
	Folin	3.50	2.0	5.5	2.00	100.0
16947	Benedict	3.64	2.0	5.60	1.96	98.0
	Folin	3.20	2.0	5.65	1.95	97.5
16882	Benedict	3.44	2.0	5.35	1.91	95.5
	Folin	3.50	2.0	5.60	2.10	105.0
16910	Benedict	3.30	2.0	5.4	2.1	105.0
	Folin	3.40	2.0	5.6	2.2	110.0
16951	Benedict	2.3	2.0	4.25	1.95	97.5
	Folin	2.6	2.0	4.70	2.10	105.0
Guinea Pig (1)	Benedict	2.7	2.0	4.8	2.1	105.0
	Folin	2.5	2.0	4.7	2.2	110.0
Rabbit (10)	Benedict	2.8	2.0	4.2	1.4	70.0
	Folin	2.9	2.0	4.9	2.0	100.0
Horse (4)	Benedict	3.4	2.0	5.4	2.0	100.0
	Folin	3.4	2.0	5.4	2.0	100.0
TB. dog	Benedict	1.3	2.0	3.3	2.0	100.0
L. O. B.	Folin	1.6	2.0	3.3	2.0	100.0

TABLE III

THE RELATION OF URIC ACID TO THE OTHER NITROGEN CONSTITUENTS OF THE BLOOD:
THE COMPARISON OF BENEDICT'S NEW AND FOLIN'S NEW COLORIMETRIC TESTS:
The Results on a Mixed Benedict—Folin Denis Reagent.*

NO. BLOODS TESTED	DIAGNOSIS	TOTAL NITROGEN	NON-PROTEIN NITROGEN	UREA NITROGEN	AMINO-ACID NITROGEN	PRE-FORMED CREATININE	TOTAL CREATININE	REST N.	URIC ACID BENEDICT	URIC ACID FOLIN	DIFFERENCE BENEDICT-FOLIN'S	*BENEDICT AND FOLIN	DIFFERENCE FROM BENEDICT
8	Incipient	2835.0	27.45	12.25	7.12	1.33	4.74	7.25	3.92	3.82	0.10	3.86	0.06
5	Mod. Advanced	3021.8	24.58	11.70	6.71	1.28	4.40	6.15	3.37	3.30	0.07	3.34	0.03
5	Far Advanced	2954.0	26.46	11.3	6.85	1.38	4.79	6.76	3.27	3.29	0.02	3.47	0.20
3	Normal	3366.6	32.9	13.03	7.3	1.40	4.9	10.9	3.60	3.53	0.07	3.55	0.05
3	Nephritis	1740.0	37.96	17.46	7.40	1.70	5.2	11.3	3.63	3.62	0.01	3.67	0.04
1	Gastric Carcinoma	3300.0	27.8	12.9	5.6	0.80	3.34	7.5	4.0				
1	Rheumatic Fever	1330.0	44.0	28.1	8.8	1.40	5.0	0.0	2.1	2.1	0.0	1.95	0.15
1	Acute Articular Rheumatism	2120.0	38.8	15.5	8.3	1.50	4.8	13.3	3.6	3.2	0.4	2.96	0.64
1	Asthma	3460.0	33.4	16.7	7.0	1.10	5.0	8.0	4.0	3.7	0.3	4.0	0.0

*The New Folin technic was used with a reagent containing three parts of the Folin and Dennis and one part of Benedict's arsenic tungstic acid reagent. The results given in the table are the average of the number of bloods given in Column I.

TABLE IV

RESULTS OBTAINED BY ADDING (1-3-5) TRIHYDROXYPHENOL (PHLOROGLUCIN) (EQUAL TO 3 MG. PER 100 C.C.) TO BLOODS IN URIC ACID DETERMINATIONS

INITIALS AND CASE NO.	TECHNIC USED		URIC ACID IN BLOOD	APPARENT URIC ACID CONTENT	COLORIMETRIC INCREASE CAUSED BY ADDING PHLOROGLUCIN	PER CENT INCREASE
I. G. 17018	Benedict	New	2.67	2.86	0.09	0.371
	Folin	New	2.60	2.70	0.10	0.385
C. T. 17017	Benedict		3.69	3.97	0.28	0.759
	Folin		3.40	3.55	0.15	0.441
N. Gears 17016	Benedict		2.76	2.96	0.20	0.724
	Folin		2.70	2.93	0.23	0.851

TABLE V

RESULTS OBTAINED BY ADDING EQUAL PARTS OF TYROSINE AND ASPARTIC ACID (EQUAL TO 7 MG. PER 100 C.C.) TO BLOODS IN URIC ACID DETERMINATIONS

INITIAL AND CASE NO.	TECHNIC USED		URIC ACID IN BLOOD	APPARENT URIC ACID CONTENT	COLORIMETRIC INCREASE CAUSED BY ADDING AMINOACIDS	PER CENT INCREASE
C. T. 17017	Benedict	New	3.69	3.65	−0.04	−0.17
	Folin	New	3.40	3.40	0.00	0.00
M. Gears 17016	Benedict	New	2.76	2.66	−0.10	−0.36
	Folin	New	2.70	2.80	0.10	0.37
I. G. 17018	Benedict	New	.2.67	2.30	0.37	1.38
	Folin	New	2.60	2.60	0.00	0.00

with double the amounts of amino-acids present as found in normal bloods, only a slight increase in color was obtained by either reagent.

From the results of Table II, the fact is shown that most animals have a small amount of uric acid in the blood as determined by the newer methods of Benedict or Folin, although Brown and Raiziss[4] found the opposite results by the old Folin-Wu technic. As Benedict has shown that the Dalmatian dog was the only animal besides man and the higher apes to excrete uric acid, it is evident from these results that there must be disintegration of uric acid before excretion. More recently Folin has shown this destruction to take place in the kidney cells and to vary in different individuals.

It has been demonstrated above, that very accurate determinations of uric acid can be made on one-half to one c.c. of blood, so that at the present time, work may be done on exogenous and endogenous uric acid metabolism in any phase of any disease.

From the results given on human blood in Tables I, II, and III it would seem that the question raised by Benedict as to the threshold for normal blood might be answered. The total and nonprotein nitrogen constituents of the bloods in Table III are normal in many cases while the uric acid content is from 3 to 4 mg. per 100 c.c. of blood. Therefore, it is evident that this ''normal level'' should be raised from 1 to 3 mg. to 1 to 4 mg. of uric acid per 100 c.c. of blood.

Tables I and III show the uric acid content of bloods in tuberculosis. It must be remembered that the plasma has about twice as much uric acid

concentration as the corpuscles and that plasma is increased in advanced tuberculosis from 55 per cent to 70 per cent of the whole blood. However, most tests give results of uric acid content about normal in all phases of this disease, with a tendency to decrease as the disease becomes far advanced. This therefore means a slight actual decrease as well as a slight relative decrease in advanced tuberculosis.

SUMMARY AND CONCLUSION

1. The Folin-Wu test does not give results equal to the total amount of uric acid in the blood.

2. Folin's and Benedict's latest methods give the same results and each give a fairly accurate recovery of uric acid added to human or animal bloods.

3. Polyphenols and amino-acids in quantities double those found in normal bloods do not make appreciable error in uric acid determinations by the Folin or Benedict methods.

4. The normal level for uric acid in the blood should be raised from 1 to 3 mg. to 1 to 4 mg. per 100 c.c. of blood.

5. Uric acid content of bloods in all stages of tuberculosis is about normal with a very slight actual as well as relative decrease in the far advanced stages.

BIBLIOGRAPHY

[1] Benedict, S. R.: Jour. Biol. Chem., 1922, li, 187.
[2] Folin, O. and Wu, Hsien: Jour. Biol. Chem., 1919, xxxviii, 100.
[3] Folin, O.: Supplement IV to System of Blood Analysis, Jour. Biol. Chem., 1922, liv, 153.
[4] Brown, H. and Raiziss, G. W.: JOUR. LAB. AND CLIN. MED., 1922, vii, No. 2, p. 129.
[5] Benedict, S. R.: Jour. Biol. Chem., xxxiv, 233.
[6] Rogers, H.: Jour. Biol. Chem., 1923, lv, 325.
[7] Folin, O. and Denis, W.: Jour. Biol. Chem., 1913, xiii, 469.
[8] Benedict, S. R.: Jour. Biol. Chem., 1915, xx, 629.
[9] Meyers, Victor C. and Fine, M. S.: Blood in Health and Disease, 1915, p. 14.
[10] Pucher, G. W.: Jour. Biol. Chem., 1922, lii, 317.

BETTER POSTMORTEM SERVICE*

By H. E. ROBERTSON, M.D., ROCHESTER, MINNESOTA

TALKING about postmortem service to a group like this is a good deal like bringing coals to Newcastle. You are, all of you, primarily or secondarily interested in good postmortem service. In fact, for some of you it is perhaps one of the main objects of work, although it is often not considered directly applicable to the work of a clinical pathologist. However, it is a subject in which every one of us finds certain phases of interest at times, and these phases I want to discuss with regard to the points that appear to be especially worth while.

One of the fundamental factors with regard to death is the obligation which *must* be assumed by any physician or group of physicians under whose care a patient dies. The contractual relation which exists between a patient and his physician is recognized far and wide, both legally and morally. The physician agrees to give his best service; the patient agrees to submit himself to the physician, and afterward to pay a reasonable fee. This contractual relation is so prominent that sometimes we are inclined to think it is the only one. There is another relation, however, that is more fundamental, and which is not so easily recognized, and that is the obligation that is brought on every physician or every group of physicians by the death of a patient under their care. When once that obligation is sensed, the most fundamental factor that has to do with the progress of the practice of medicine is sensed. When a patient dies, a great debt is owed to humanity in order that the patient shall not have died in vain, and it is our duty as practicing physicians (not necessarily as pathologists) to see that the utmost possible benefit to our future patients, to the physician, and to humanity as a whole, shall come from this death. Why do we not all assume that obligation in a greater degree? Why do we not all obtain permission for examination, and review the deaths in 100 per cent of our cases? More simple still, why do we not all *ask* for permission in 100 per cent of cases? A number of reasons will readily occur to all of you, but I shall mention one of the most potent which may not have occurred to all of you. It has to do with the recognition of the very obligation I refer to. It has to do with the fact that the physician himself has not had "sold" to him the idea of the necessity and value of postmortem examinations. You will say that is certainly not true, for what physician would say that the postmortem examination is not valuable? Yes, the postmortem examination on *somebody else*. The other day a medical student from one of our leading medical colleges, in his last year in the medical school, was approached on the subject of postmortem

examination on his father. He said, "I hope you will not insult me by asking permission for an examination in the case of my father." This is not a rare instance. Such "insult" would be inferred by an alarming percentage of physicians throughout the country and just so long as this attitude maintains, we shall have a barrier of inertia against the obtaining, and against the enthusiasm for, postmortem examinations in our hospitals. We do not need to bring it back to the usual pleas that "people don't want examinations," and "we must not offend them by asking for it," and "these people are so peculiar they don't like to have examinations." Let us bring it where it belongs, in many instances, and that is right up against the attitude of the physician himself. You can test it out. Test it out in your own mind. Test it out in the minds of your fellows. Are you going to permit a postmortem examination in the event of the death of one of your family? If you are not, I tell you now you have no right to ask anybody for permission to perform an examination on the bodies of their loved ones. I feel very strongly about this, because I have lived for a good many years (fifteen) under the mistaken and somewhat youthful enthusiasm that the doctor's interest and personal responsibility for the postmortem examination was a thing to be taken for granted, and I found it an extremely rude surprise when I began to investigate the subject closely, to ascertain that this interest not only could not be taken for granted, but that far and wide in a subtle way there was a feeling among doctors that "this is all right for poor Yorik in the city hospital; it may be all right for the fellow who is picked up in the street, but for one of the members of my family, for my friends, this is an insult." I wish to emphasize that every physician who has a person die under his care is in honor bound, and under obligation to humanity, to ask permission for a most thorough painstaking examination, made with the utmost possible respect, and with the idea that that death is going to be of value to humanity because of the things revealed. When doctors say to us, "You get permission here at the Mayo Clinic in 85 or 90 per cent of your cases, of course, because you have here a different class," they raise the red flag to the bull about as quickly as in any way I know, because we do not see very much difference in the people coming here and the people coming to other physicians. One reason why we get permission is because we are willing to grant permission on our own relatives; that is, we have that fundamental attitude. I want to stress this very point, that you have got to "sell" the idea of postmortem examinations to the doctors themselves. Fundamentally, they are not convinced. Another reason for our success is because we approach the relatives with the idea that we think this is the thing to do, and we think this is the way in which we are going to be able to serve humanity a little better.

A few points about the practical performance of a postmortem should be emphasized. One of them is, if a postmortem is worth doing at all, it is worth doing well. You are committing, in spite of what I have said, a certain sacrilege on the bodies of dead persons by the performance of a postmortem; and it does not make any difference who they are. It is obligatory upon you, therefore, that everything you do should be done most thoroughly.

Consequently you should learn to perform a postmortem properly, and if you do not know how, find out; you can do this even by reading. Anybody can make a good postmortem examination. There is no excuse for doing a poor one. If almost any doctor can be made a surgeon (and with all due respect to the surgeons, it would seem almost as if that were true), then, certainly almost any pathologist can learn to do a postmortem; and yet a postmortem well done, not necessarily expertly but carefully and systematically, should be much more common than it is. Another thing: take the relatives into your confidence; explain to them just why you want to make the examination, and after you finish, show them what you found. They will be tremendously interested. Make it of value to them; tell them what the chances are, so far as their inheritance is concerned, and with regard to vascular disease, discuss with them the pathogenesis that exists in the relative who has passed away; discuss with them the meaning of these tuberculous lesions in the chest; discuss the manner of living, what it may have meant to the deceased and may mean to the living relatives. Discuss the causes of death, so far as you can; lay your cards on the table; you will find that you will be met a good deal more than half way. You say, "I don't want to talk to this poor wife; she is all broken up." The one thing she wants to talk about is her husband's sickness, or her child's sickness; such discussion is the outlet for her emotion, and you are doing her a service. I know of nothing more comforting to relatives than to discuss frankly and freely the conditions that brought about the death of their loved ones. In the course of three years of discussions with relatives I have not seen one who did not feel better because he or she has had a chance to talk about these things openly. The screen that we draw between the patients and our practice should be torn down almost completely. If we do not tear it down, it will be torn down for us. People are getting more and more information concerning these subjects, and we must take this into consideration.

Another point: be sure to cooperate with the undertaker, who is in a position to render the most important opposition. Cooperation can be easily arranged. When I came to Minneapolis a number of years ago, I found that in order to make an examination one had to make it before the undertaker got there. If he could possibly prevent it, he would. Before I left the University of Minnesota at Minneapolis I had the unique experience of being called up by an undertaker and asked to make an examination because he felt the relatives should know the cause of death. In this particular case he had obtained permission for it, the physician in charge not having anything to do with it. Such a state of affairs is easily brought about. In the first place, remember that the undertaker, and the pathologist also, assume certain obligations toward the decent repair of the body; consequently, the pathologist should learn how to prepare a body after death. There is nothing complicated about this procedure although the undertaker's charges sometimes make one think differently. On the one hand certain measures on your part will aid the undertaker, and on the other hand, other measures may make it almost impossible for him to do a decent job. Again and again I have seen

undertakers frothing at the mouth with the condition in which the remains were left, and they were expected to bear the brunt of the difficulty, simply because the physician did not understand his task. In many instances I have called in the undertaker and explained to him that I wanted to do this or that, and asked his help. In other words, I have treated him as I would a cooperator in any other form of effort. Help him in his work and you will find very soon that he is willing to help you, because he wants to work with you instead of working against you.

Another important point is the preservation of gross specimens. Do not ask your staff to be interested or excited about a lot of dirty, foul-smelling, bloody stuff that you bring before them. The physician who is not accustomed to postmortem work does not like to get his hands or his clothes, or anything, dirty and covered with blood. This appears strange to some pathologists, but nevertheless it is true. The physician does not like to smell these specimens. This seems strange too, but it should be remembered that he was not brought up in such an atmosphere. There is no excuse for dirty, bad specimens. If this idea of selling postmortem examinations to the staff is to be developed, specimens must be preserved in a decent, presentable manner. Elaborate equipment is not necessary. Buy a large stone crock and put a cotton band around the lid, thus making it fairly tight, and in the stone crock float tissue attached to a cork by a string. The solutions, which may be prepared according to several formulas, we are now using in such a manner as not to require alcohol. Good specimens will be obtained which the surgeon will be glad, instead of sorry, to look at. One doctor says, "I have not had time yet to go into the question of gross specimens," and yet he is doing postmortems right along and showing the surgeon the result of his work. The only way to make postmortems popular is to show the surgeons specimens which they can appreciate, and which do not disgust them by their appearance. Remember that it is a simple matter to preserve specimens. Mounting in expensive glasses, and in an expensive solution is not necessary; they can be put in cheap jars with a piece of fiber label tied to them. Pieces of sections of every bit of tissue should be preserved for microscopic study. Some of the most amazing facts have been disclosed through the study of microscopic sections. One more point: insist that among your colleagues there is an open, free and frank discussion of this particular postmortem examination. It does not make any difference if you make only one examination every three months. Have a meeting of the staff on the poor fellow and go over his case in extenso. If there is anything about that case that the clinician did not understand, make him understand it. If there is anything that comes out of that case that will lead him or you to give better service to the next particular patient who has that disease, bring it out. If there is any way in which you can teach the surgeon to come out and face openly that he performed an appendectomy, and the patient died from peritonitis, and the chances were that the abdomen was infected at the time of operation, you have done a great work. Of course, you have to be a little careful how you put it. The surgeon may be a bigger man than you are;

nevertheless, if there is any way (I am overemphasizing this, of course) by which that postmortem can be made to teach the physician to face the issue openly, you have taken a tremendous step in advance. Clinicians and surgeons do not like to face the issue. Even pathologists do not like to admit they are wrong, and in this respect they are almost as human as the rest of the doctors who do not like to admit they are wrong; they prefer in some way or another to gloss over that thing we call "reputation." Keep it among your own crowd and do it quietly, and the next time a like case comes up and you have to face the issue, you will be a good deal more willing to say, "Yes, that man came to me. I wish I had not taken out his appendix; I knew he had a weak heart, but I didn't pay enough attention to an examination of his heart, but the next fellow that comes to me like that is going to have a good examination of his heart." When you can get results like that you are accomplishing something and your patient will not have died in vain.

(For discussion, see page 505.)

(For discussion, see page 505.)

Boylston Medical Prizes of Harvard Medical School

The Boylston Medical Prizes, which are *open to public competition*, are offered for the best dissertation on questions in medical science proposed by the Boylston Medical Committee. The Committee is appointed by the President and Fellows of Harvard College. The names of the Committee appear in the annual catalogue of the Harvard Medical School.

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Dissertations entered for this prize must be in the hands of the Secretary on or before December 31 of the year in which the prize is offered.

Each dissertation must bear, in place of the author's name, some sentence or device, and must be accompanied by a sealed packet, bearing the same sentence or device, and containing the author's name and residence within.

Any clew by which the authorship of a dissertation is made known to the Committee will debar such dissertation from competition. Previous publication of the work, if in form to give a clew to authorship, will debar from competition.

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All unsuccessful dissertations are deposited with the Secretary, from whom they may be obtained, with the sealed packet unopened, *if called for within one year* after they have been received.

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1. That the Board does not consider itself as approving the doctrines contained in any of the dissertations to which premiums may be adjudged.

2. That, in case of publication of a successful dissertation, the author be considered as bound to print the above vote in connection therewith.

The address of the Secretary of the Boylston Medical Committee is Dr. Henry A. Christian, Peter Bent Brigham Hospital, Boston, Mass.

LABORATORY METHODS

MODIFICATIONS OF THE SAHLI HEMOGLOBINOMETER[*]

By Wm. C. Thro, M.D., New York City

SINCE the determination of the hemoglobin is one of the most important tests made on the blood and all laboratory workers have difficulties with different types of hemoglobinometers, it seemed that the following experiments might be of interest.

In spite of the fact that it is frequently stated that it would be better to express the amount of hemoglobin in grams per hundred, it is the universal custom to express it in percentage. It seems then that the standard of comparison may be a mixture of bloods of normal healthy human beings or a blood in which the hemoglobin is normal as determined by some chemical test like the oxygen combining power.

In working with classes of medical students for a number of years, it has been found that a more accurate comparison can be made with brown colors than with shades of red. The reds are used in hemoglobinometers like those of Fleischl, Fleischl-Miescher and Dare.

Our experience has been that the Dare instrument is easy to use and is the one to be preferred when fair accuracy is desired. It is rather an expensive instrument, however, and occasionally the two pieces of colored glass used as a standard are not well matched, and a number of students reading the same blood will get results varying from 5 to 10 per cent.

One of the chief faults of the Sahli is that the color standard fades, a conclusion arrived at by many laboratory workers. On opening a number of the standard color tubes (made by several manufacturers) and taking measurements of the bore, we were astonished to find variations from the required 7 mm., of from 7.3 to 6.6 mm. as measured by a Brown and Sharpe metric inside micrometer. These are the actual measurements of the bore of fourteen standard color tubes: 6.65, 6.71, 6.74, 6.81, 6.85, 6.86, 6.92, 6.93, 6.95, 6.99, 7.0, 7.03, 7.05, 7.35. However, when a local glass manufacturer made us some of these standard color tubes, it was found that the variations in the bore varied more than in the above. This only shows that the preparation of such apparatus, where accuracy is required, cannot be turned over to people who are not acquainted with the importance of a variation of a millimeter in the thickness of a colored solution.

Since we believe that, if properly made, the Sahli is one of the best hemoglobinometers, we proceeded with the idea that it could be made more accu-

rate if certain alterations were made. In the instrument at present on the market, the color tube and the graduated tube are 7 mm. apart, but a more accurate reading could be made if the two tubes were nearer together. It is therefore advisable to have the two tubes separated only by a blackened partition about 1 mm. thick. In using the Sahli, nearly everyone, when making the comparison, covers the upper portions of the tubes with the hand so that only a small length of both is visible. An instrument was therefore constructed in which the entire length of both tubes was hidden except a portion that is visible through an aperture 16 mm. × 16 mm. The accompanying figure is an outline of such a model. There is a small piece of ground-glass at the back. The base is of lead to make the instrument more stable.

The tube which is to contain the standard color is made like a small test tube with accurately gauged bore exactly 7 mm. in diameter and closed with a rubber stopper. It was admitted by one manufacturer, when he was shown
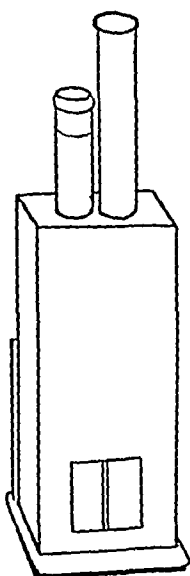
Fig. 1.

a very dark color tube, that careless heating of the glass tube to seal it causes a darkening of the solution. If the color changes in the stoppered tube, the material may be easily poured out and the tube cleaned and dried, sterilized and refilled with fresh 1 per cent acid hematin stock. We have found that if the comparison color mixture is made up and put into a brown glass bottle which is kept in the dark, that the color will not change in four years.

Make the standard hematin solution of:

Blood oxalated	1 c.c.
HCl tenth normal	30 c.c.
Glycerine	69 c.c.

Sahli finally used a mixture of 1 per cent blood in water.*
Use the blood of an individual on whom the oxygen combining power has been determined or pool the blood of several healthy individuals.

*Sahli's Diagnostic Methods, Trans. by N. B. Potter, 1911, Edition 2, p. 749.

Sahli used as a comparison solution the acid hematin of the blood of individuals who evidently had a very high hemoglobin content, since with the instruments that came from Switzerland it was necessary to make a correction. For example, a reading of 65 equals 100 per cent.

For six years we have conducted the following experiment in the class in clinical pathology.

With the apparatus that comes with the Sahli hemometer, each student makes a 1 per cent acid hematin solution of his blood and this is poured into a flask. After all the solutions are added, the flask is well shaken and a portion of the mixture taken by each student and placed in the color tube and a hemoglobin determination made on his or her blood. (Before performing the experiment the student had some practice in the use of all the common hemoglobinometers.) These are the results:

YEAR	STUDENTS	AVERAGE
1918	50	94.0%
1919	37	99.0%
1920	40	100.0%
1921	58	95.0%
1922	53	98.8%
1923	55	100.8%

A variation of from 4 to 5 per cent is permissible in the reading and it is to be noted that the percentage comes very close to 100 per cent. With some very robust students, who had also a high red blood cell count, the percentage ran considerably above 100 and with others it was below 90, but it is interesting to note that the average in the six years was very close to 100.

A COLLECTING TUBE FOR SERUM*

By William G. Exton, M.D., New York City

IT is often necessary or desirable to delay the making of Wassermann and other tests of sera until some practicable or convenient time after collection of the specimen. It is also frequently impossible or impracticable to keep the specimens on ice during this delay. When the time that elapses between collection and testing is not prolonged, or when the sample of blood does not tend to hemolyze, the aseptic collection of blood by means of the vacuum ampule, introduced by Keidel for this purpose, is efficient and convenient. When, however, it is desired to provide for the satisfactory preservation of sera for days at a time and at room temperature in order to meet conditions incidental to getting specimens from remote localities to the laboratory through the mail, such as the conditions incidental to the Prudential Longevity Service, some other and more reliable method of preservation was found necessary.

Experiments showed that sera which were separated immediately from

blood collected under aseptic conditions would not, of course, hemolyze, and also that a week or ten days, even a month or more, might elapse under mailing conditions without the sera becoming anticomplementary. After trying out a number of devices designed to accomplish the separation of serum conveniently, the one here illustrated was adopted and has proved very satisfactory in practice.

Fig. 1 shows the serum collector packed in a mailing case and ready for shipment. After removing the collector from the mailing case detach the small test tube (Fig. 3), which acts as a needle cover, and transfer the rubber nipple from the closed glass end slipping it over the mouth of the small test tube. Remove the stylus with the small attached cork from the needle point, and collect the blood in the usual way as with a Keidel tube. The tube with the blood may then be centrifuged (if a centrifuge is available) or left standing

Fig 2

Fig 3

EXTON-COMER SERUM COLLECTOR

PAT APPL FOR
Fig 1
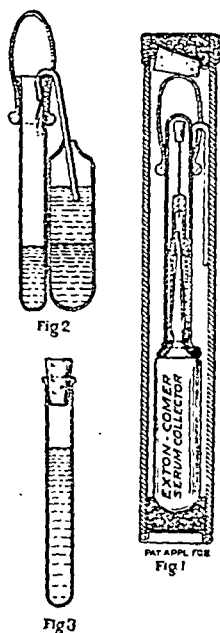
in an upright position in a glass of ice water or at room temperature. When the clot forms and the serum separates, snap off the stem of the ampule at the small file scratch.

Fig. 2 shows the manner in which the separated serum is transferred from the collector to the small test tube. Press the rubber nipple so as to force the air out of the siphon tube, and, taking care not to disturb the blood clot, place the longer tip of the siphon tube near the bottom of the layer of clear serum. When pressure on the nipple is released the serum will be drawn rapidly into the small tube, which is stoppered with the cork provided in the container. It is then ready for mailing. For sterile collections the rubber nipple, siphon tube and rubber stopper may be boiled.

All of these manipulations are simply and easily carried out, but care should be taken to avoid agitations likely to cause blood cells and serum to mix. The siphon arrangement was made by Harris Comer, of the Steel Glass Company, of Philadelphia, who is in a position to supply complete outfits ready for mailing.

A CONVENIENT OUTFIT FOR THE SCHICK TEST*

BY AUGUSTUS WADSWORTH, M.D., NEW YORK

WHEN the Schick test was first adopted in this country, none of the out-
fits for its administration were considered sufficiently reliable for accu-
rate or general, state-wide use. Diphtheria toxin for this test is diluted so
that 0.1 c.c. of the diluent will contain one-fiftieth M.F.D., or one-fiftieth of
the quantity that will kill a guinea pig weighing 250 grams in about four days.
One-tenth of a cubic centimeter of this dilution is injected intracutaneously
with a fine needle. The toxin is not only very labile and subject to rapid
deterioration, but such minute quantities must be so accurately measured that,
in sending an outfit to different parts of the state, it was necessary to have
one which, besides being convenient, would remain serviceable for some time.

In 1916, Miss Tula Lake Harkey undertook the problem of devising a new
outfit before she went overseas as chemist to Base Hospital No. 33. While in
England, she developed cerebrospinal meningitis and died, as have so many
others, in the service of her country. Work which she did on standardizing
an outfit was so successful in leading to practical results that I wish to record
her observations together with a description of the outfit, which has now been
in use, with very slight modifications, for nearly seven years.

In her investigations, Miss Harkey found that the number of drops in
each cubic centimeter of a fluid delivered from different pipettes varied mark-
edly, but when the same pipette was used the variation was not so great; and
when a small pipette with a fine point was standardized, the drops were very
nearly constant. While she was engaged in her work, Donald[1] and Walker[2]
published methods for measuring drops by the use of standard solutions of
hydrochloric acid and sodium hydroxide. Following their suggestions, stand-
ard solutions of 4N HCl and N/100 NaOH were used as follows: The hydro-
chloric acid solution was drawn into the pipette, and then one drop of the
hydrochloric acid solution falling from the pipette was titrated against the
sodium hydroxide solution. Three titrations were necessary for each pipette,
and the average of these was taken. With accurate solutions the volume of
the drop of acid was found to be one-four-hundredth of the volume of sodium
hydroxide used to neutralize the acid. From this volume was calculated the
amount of physiologic salt solution required to dilute the toxin so that 0.1 c.c.
of the dilution would contain one-fiftieth M.F.D of the standard toxin.

It was early decided that a second pipette would increase the efficiency
of the outfit by providing a reserve in case of possible breakage of the tip of
one, and by allowing a second dilution to be made when the volume of sterile

salt solution was replaced in the graduated bottle. Later, the advantages of preparing some outfits with more bottles of salt solution became apparent.

Any outfit designed to measure so small a quantity of material with accuracy necessarily requires more careful technic than the average physician is ordinarily obliged to employ. This explains why, at first, some have thought it too complicated. Practice and a trial of other outfits soon change this opinion, especially as the results obtained are most gratifying. From the standpoints of maintaining potency and enabling the laboratory to meet the unequal demands of state-wide distribution, it has proved most satisfactory. The methods employed in its preparation and directions for its use follow.

The Outfit.—The outfit consists of a small vial of undiluted diphtheria toxin, a pair of standardized capillary glass pipettes for measuring the toxin, one, three or eight small bottles containing the exact amount of salt solution necessary to give the required dilution when one drop of toxin from the accompanying pipette is added, and a small sterile tube for use in preparing the control test. The outfit containing one bottle of salt solution is designed for general use; those containing three or eight bottles are suitable when many persons are tested at one time.

Preparation of Capillary Pipettes.—Five millimeter glass tubing with a rather thick wall is broken into four-inch lengths with ends slightly annealed, thoroughly cleaned and dried, and drawn out to form two capillary pipettes with very small bore. Each pipette is standardized by titrating the drop of 4N HCl it delivers with approximately N/100 NaOH, standardized against the acid.

Standardization of Hydrochloric Acid and Sodium Hydroxide Solutions.— The hydrochloric acid and sodium hydroxide solutions are prepared so that the HCl is 4N and the NaOH approximately N/100. The sodium hydroxide solution is then standardized against the hydrochloric acid solution, 1 per cent aqueous solution of alizarine sodium sulphonate being used as indicator. To ensure accurate results two N/10 HCl dilutions are prepared from the 4N HCl and three 2 c.c. samples of each dilution are titrated with NaOH. The titrations must agree within 0.1 c.c. In titrating, the volume is made up to about 70 c.c. with water, and one drop of indicator is added. The end-point is determined by comparison with a control flask containing water and indicator only. This flask is prepared at approximately the same time as the titration flask, as there is some change in color on standing. The value of 1 c.c. of 4N HCl in terms of NaOH is determined as in the following example:

Average reading 2 c.c. N/10 HCl = 19.962 c.c. NaOH
1 c.c. N/10 HCl = 9.981 c.c. NaOH
1 c.c. N/10 HCl = 40 c.c. 4N HCl
1 c.c. 4N HCl = 399.25 c.c. NaOH

Standardization of Capillary Pipettes.—The volume of one drop of 4N HCl delivered by the pipette is determined by titration with the standardized NaOH solution. If the variation in the burette readings is not more than 0.05 c.c., three titrations are made and the average is taken, if 0.1, five titrations, and if greater, the work is repeated. The pipette is filled half-full, held

in a vertical position, gentle pressure is exerted upon the upper end until the drop starts to form, when the pressure is removed and the drop is allowed to fall unaided. The first one or two drops are discarded.

Each standardized pipette is cleaned, labeled with the number given it on the record card, and placed in a plugged tube with a cotton guard at the bottom. After sterilization in hot air the pipettes are paired. The volumes of salt solution required must be the same, within a 0.05 c.c. variation, for both pipettes.

The volume of 0.85 per cent salt solution used for diluting a drop of toxin delivered by the pipette so that a final dilution of one-fiftieth M.F.D. in 0.1 c.c. is obtained, is computed as follows:

Assume that the drop of 4N HCl delivered by the pipette is neutralized by 2.9 c.c. of the NaOH solution standardized above, and that the M.F.D. of the toxin to be used is 0.003 c.c.

$$\text{Vol. drop:} \quad 1 \text{ c.c.} = T \text{ (titration of drop with NaOH)} : 399.25$$

$$\text{Vol. drop} = \frac{1}{399.25} \times T$$

$$\frac{1}{10} \times \frac{1}{\text{Vol. salt}} \times \text{Vol. drop} = \frac{1}{50} \times 0.003$$

$$\text{Vol. salt} = \frac{5 \times \text{Vol. drop}}{0.003} = \frac{5}{0.003} \times \frac{1}{399.25} \times T$$

$$= 4.174T$$
$$= 4.174 \times 2.9$$
$$= 12.104 \text{ c.c.}$$

The volume of salt solution required to dilute the drop from this pipette is then 12.1 c.c. General formula:

$$\text{Vol. of salt solution required} = \frac{5}{\text{M.F.D.} \times S} \times T \text{ in which}$$

S = value of 1 c.c. of 4N HCl in terms of NaOH
T = value of drop of 4N HCl in terms of NaOH

The bore of the pipettes selected should be such that the drop falls easily and unaided except for the initial pressure to start its formation. A drop of the proper size, when toxin with an M.F.D. of 0.003 is used, requires from 11 c.c. to 14 c.c. of salt solution.

Diphtheria Toxin.—A well stabilized toxin with an M.F.D. of approximately 0.003 to 0.004 is selected. Before being bottled, it is restandardized by the subcutaneous test. Guinea pigs weighing between 250 and 280 gm. are used, at least 75 per cent of which should die with typical lesions in somewhat less than ninety-six hours. If these tests are satisfactory, two guinea pigs are injected intracutaneously with $\frac{1}{50}$, $\frac{1}{250}$ and $\frac{1}{500}$ M.F.D. The first dilution should induce a very marked reaction, the last a reaction corresponding to a definite Schick reaction in a person. Tests upon laboratory workers of known susceptibility or immunity are valuable as controls.

Bottling of Toxin.—The toxin is bottled in approximately 0.75 c.c. amounts in 1 c.c. amber vials which tests have been shown to be practically neutral. During the process the toxin is protected from light and warmth as much as possible.

Assembling of Outfits, Including Bottling of Salt Solution.—Before the salt solution is dispensed, the pipettes, and labels for the bottles giving the numbers of the pipettes and the volume of salt solution required, are placed in wooden boxes and each outfit is given a serial number. The required volumes of sterile 0.85 per cent salt solution are filled into small bottles, each of which is closed with a rubber stopper and placed immediately in the corresponding outfit. The filled material is tested for sterility. The bottles are capped with fish skin, examined for defects, and marked with a diamond pencil to indicate the level of the salt solution in order that possible leakage may be detected readily, or that the bottle, properly rinsed and sterilized, may be refilled and used again with the second pipette. The outfits are then completed except for the toxin, which is added immediately before they are placed in the cold room.

Time Limit.—When an outfit is to be sent out, the "Return date" is entered on the label of the box. In the case of a toxin standardized for the first time for use in the Schick test, this date is three months later than that of the standardization test. About a month before the date of expiration, the toxin in the small bottles is retested. If no deterioration is shown, the time limit on the outfit is extended two months. This procedure is repeated at bimonthly intervals.

In each outfit a circular is enclosed, describing the material and equipment and giving directions for use, from which the following essential points are taken:

Precautions to be Observed.—Do not use the outfit without first reading carefully the directions, which should be followed exactly. Keep the outfit in an ice box—the toxin deteriorates rapidly at room temperature or in the light. Always make sure that the number of the pipette given on the bottle of salt solution and the number on the capillary pipette correspond. Do not use a pipette with a broken or nicked tip as any change in the tip will alter the size of the drop. See that the level of the salt solution corresponds to the line on the bottle. Do no use any part of one outfit with another outfit. Never use the diluted toxin when it is more than twelve hours old.

Preparation of Dilution.—Under sterile precautions remove the capillary pipette from its container and by gentle suction draw up from the bottle of undiluted toxin enough toxin to half-fill the pipette. Hold the pipette vertically, with the thumb and second finger, and press gently on the top of the pipette with the first finger until a drop begins to form. At once remove the finger and allow the drop—which should be discarded—to fall unaided. Repeat the procedure and allow the second or the third drop obtained in the same way to fall into the bottle which contains the exact quantity of salt solution necessary to make the correct final dilution. Never add more than one drop to the diluent. Cork the bottle and mix the contents thoroughly. Each 0.1 c.c. of the dilution contains the required dose of toxin for the test.

Preparation of Control Dilution.—Transfer part of the diluted toxin to the small sterile test tube by means of a sterile pipette—allowing no toxin to touch the upper part of the tube—or by pouring and then flaming the top of the tube thoroughly to destroy adherent drops of toxin. Plug and immerse in boiling water for three minutes.

SUMMARY

A convenient outfit for the administration of the Schick test is described and a detailed account is given of a method of preparing it which has been in use for several years.

Outfits made and assembled as described have been distributed in large numbers during a period of nearly seven years and have been found highly serviceable and reliable.

REFERENCES

[1]Donald, R.: A Method of Drop-Measuring Liquids and Suspensions. Lancet, December 4, 1915, ii, 1243.

[2]Walker, E. W. A.: An Application of Drop-Measuring the Widal technique. Lancet, September 23, 1916, ii, 552.

ELECTRIC HEATED FUNNEL FOR FILTERING MEDIA[a]

By Emil Weiss, M.D., Chicago, Ill

THE hot water funnels in use for filtering agar and gelatin are slow, wasteful and hard to keep clean. Filtering in the autoclave requires very much time, and excessive heat is usually applied to the media during this period of time. We have made a funnel similar to the hot water funnel, but used electricity as our source of heat. Fig 1 shows the funnel complete
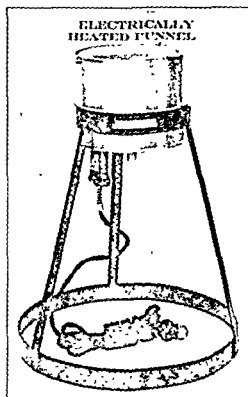
Fig. 1.

as we are now using it. Fig. 2 is a cross-section of this funnel. The wires (w) are placed on the bottom. The inside of the air space (x) is enclosed with asbestos (A) to prevent loss of heat and also to keep the outside aluminum covering from getting too hot. The funnel is made of aluminum. A glass funnel can be put inside of this if desired. The temperature inside

the funnel is 90° C. Fig. 3 shows the arrangement of the heating wire at the bottom of air space.

We have been using such funnels for twelve months in the medical school and hospital laboratories. The temperature is constant; there is
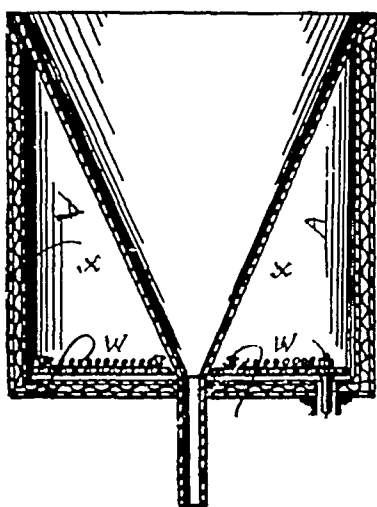
Fig. 2.
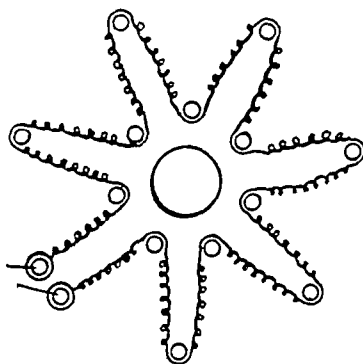
Fig. 3.

practically no waste of agar or gelatin; the working space can be kept clean as there is no boiling over of water; there is no danger of fire from exposed gas flames; the filtration process can be quickly started and rapidly performed with such an apparatus.

TRANSACTIONS

THIRD ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

June 5-7, 1924

(Continued)

Symposium on Laboratory Procedures

President MacCarty.—My object in having this little symposium is this: I do not think that all of the clinicians really appreciate what the laboratory does for medicine. They have had an idea that the laboratory was a thing apart from medicine; that medicine is merely the art of getting the patient, making him feel good and holding him—pleasing the family, in other words. Nevertheless the laboratory still has in the practice of medicine a close relationship. There is nothing vicious in the attitude of some clinicians, but they do not understand what the laboratory is trying to do and actually does accomplish for medicine. The laboratory man on the other hand has not always appreciated the physician's activities. Now, we have come into medicine since the advent of laboratory medicine, and we have spent most of our lives in laboratories, and have not come into close contact with the actual practice of medicine. In other words, we are not in the actual practice of medicine. Laboratory sciences, as you know, are quite recent, and have required that the laboratory man spend considerable time working in the laboratory; thus he has not had the opportunity to take care of patients. I think it is about time, since we have standardized so many laboratory procedures, for the physician to be more intimately familiar with the laboratory procedures and their value, and for the laboratory man to be more familiar with the art of the practice of medicine. It was with this goal in my mind that I had this symposium arranged. We have in this institution, fortunately, many men, young men all of them, who are actively in contact with patients, and who at the same time, being young, have had extensive laboratory experience; we have tried to make laboratory practice, and clinical practice one thing, and not keep them separated. The men who are on this program today are primarily interested in patients, and are also well trained in laboratory procedure. I shall call on Dr. Sanford first.

Doctor Sanford.—My few remarks are going to be very impromptu, because I have been thinking the last few days whether you are going to get something to eat, and a place to sleep, and prosaic things of that sort.

I think I will have to put a little "reverse English" on my first shot around the table, and say that I believe we do too much laboratory work now. I think the pendulum has swung a little too far. Twenty years ago we were stressing physical diagnosis and the observation of signs and symptoms. The young man coming to us now wants every laboratory test that he has ever heard of before he is going to hazard a guess as to what is wrong with the patient, or take a look at him, and sometimes it leads him into a rather bad pitfall. I want to emphasize the points that MacCarty has just mentioned. I believe that the laboratory man should also be a clinician and should wherever possible see the patient, acting somewhat as a consultant as to the advisability of doing certain tests.

Laboratory procedures are stressed to a great extent at the Mayo Clinic. In making out the history, the first man that sees the patient writes the history; he has a little yellow slip attached to the history, upon which he puts those things he wants done to aid him in mak-

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ing his diagnosis. On the slip is a list of various clinical laboratory procedures. In the few minutes at my disposal I will go down the list of things done in my section and try to make a suggestion, where I can, as to whether they are of very much clinical value. There are certain things we do routinely, and I think that probably their clinical value has become established.

Of course, we do a urinalysis, just the ordinary routine urinalysis. Patients are sent for hemoglobin, and red and sometimes white counts. Gastric analysis: I do not know really how much clinical value gastric analysis has, except to find out whether there is retention. Of course, that statement is a little strong. Then, in bacteriology I would say that clinical bacteriology, the majority of it, is rather negative. Negative results are of value, of course, in throat cases, and so on, and yet a negative finding is negative and that is all we can say about it. Our positive findings in bacteriology certainly are of great aid in diagnosis. Some of the special things we do in blood work,—the fragility test, for instance; if you have an increase in fragility, you have made a diagnosis, when coupled with the physical findings. The coagulation factors: an increase in coagulation time is of great service, usually, to the surgeon. Whether he wants to operate before something is done to alter coagulation factors is often a question to be decided by the laboratory. Plate counts, prothrombin determinations may help in making a diagnosis of hemophilia. Blood grouping: we do a great deal of blood grouping as you know, here. We could not do our transfusions without it by the methods that we use. The surgeon would be lost if we did not have on hand all the time Group IV donors ready for emergency transfusions.

As far as a Wassermann is concerned, Stokes says it is worth about fifty per cent in diagnosis, especially in the field of dermatology. I do not care to start to argue that, one way or the other. We do not do a routine Wassermann here. Much of our attitude toward the Wassermann has been influenced by the dermatologist's attitude toward it. We rather arbitrarily do Wassermanns on patients between fifteen and thirty years of age, not that age has much to do with it, except that Stokes says he can do something for the syphilitic patient between fifteen and thirty years of age, and he is not so sure about those later in life. All patients undergoing operations should have Wassermanns; the surgeons require it of their patients. Of course, we are assuming that the Wassermanns really help them in the diagnosis of syphilis, and according to Stokes it helps him just about fifty per cent. The other fifty per cent he bases on history and his own findings and physical examination. Blood culture: of course, there is nothing much to say about that. When it is positive, it makes a diagnosis. We do considerable blood chemistry; much that is negative. I think this is the age, or perhaps a year or two ago was the age, when blood chemistry was the important thing, and out of it all we have sifted some rather significant clinical procedures. The blood urea now is just about as common a clinical laboratory test as the functional test—but not quite. The functional test comes first, but it is usually now checked with blood urea. It does tell us some astonishing things at times. I used to think it was useless to do a blood urea on patients who would walk into the laboratory, but they do walk in with considerable percentages of blood urea. When the patient is in the hospital, blood urea determination often helps in the diagnosis, not as to uremia, but as to kidney function. Blood sugar: Wilder will stress that a good deal more in his talk. Examination of spinal fluids: there is very little indication for withdrawing the spinal fluid at all except for the relief of the pressure, but the laboratory procedures are often of extreme value in making a diagnosis. The stool examination: we perhaps do more stool examinations than are done in most places. This really is because for the last thirteen or fourteen years we have been very much interested in the fact that our Northern patients had amebiasis or other intestinal parasites, and while they were not of as much importance as in the South, they were of interest. About twenty per cent of our stool examinations are positive for some species of intestinal parasite. Of course, a large percentage of these probably have not very much to do with the patient's symptoms. Often we do find ova of parasites when the patient does not know that he is harboring any of the worms.

Sputum examinations: We do these at the request of the chest specialists. The skin

sensitization test: we are working with the internists on asthmas and hay-fevers, but I do not know that we are up to date, since hearing Black's paper yesterday. I want to take this occasion, if he is here, to congratulate him on the excellent piece of work that he did in that paper. I think we probably are working with a 1923 model, and he has given us the 1925 ideas.

Guinea pig inoculations: we use about one thousand guinea pigs a year. I would say that Branch considers the test of extreme importance in his diagnosis of kidney tuberculosis: and for Henderson, I think it is more in confirmation of diagnosis in tuberculosis of bones and joints, because we get the material after operation.

Sinus examinations: You perhaps know that we have been very much interested in the prevalence of actinomycosis. We have had over two hundred cases here. The examination of old sinuses leads us to the finding of the characteristic "sulphur bodies." Magath has been very successful also in finding of tubercle bacilli in tuberculous sinuses.

The examination of duodenal contents with Schneider's test, demonstrates the increase in urobilin and urobilinogen and helps somewhat in the diagnosis of pernicious anemia, if we accept the theory that the increase in these pigments means the increase in blood destruction. It does go along with it most certainly, and while I would not consider it of great value in diagnosis alone, it is confirmatory.

President MacCarty.—The next speaker, Rowntree, needs no introduction, I am sure. I am going to use him as an example of what I think the real physician should be. The first time I met Rowntree was in a pharmaceutical laboratory. I was visiting Baltimore, and he was there. I do not know that he had ever seen a patient or ever expected to see a patient. The next time I saw him he was running around the wards in Johns Hopkins Hospital, and was interested in patients. Here, where I live with him, he does both; he sees a number of patients a day, and is in the laboratory, and he can give a very practical picture of medical laboratory problems.

Rowntree's remarks have been published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE in the issue of October, 1924, page 75.

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DRS. KOLMER and STEINFIELD read a paper entitled **A Study of the Specificity of the Kolmer Complement-Fixation Test for Syphilis,** published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, x, No. 1.

DISCUSSION

Doctor Sanford.—Kolmer, as usual, has gone thoroughly into the subject. The only experience we have had statistically is in a paper that he has mentioned. In the five hundred diabetics, we might say that the Noguchi antigen did not give any more false positives than his antigen; that is, both of them gave no false positives. Incidentally, we might say that the incidence of syphilis, judging from the Wassermann reaction, and the history, was really lower in these diabetics, over five hundred of them, than is the incidence in the average run of patients that registers at the Mayo Clinic. I am not trying to argue against the statements of some men that syphilis has something to do with diabetes, but our findings do not justify such an opinion. For years we have had all our serology on patients that have been fasting, and for many reasons, we will continue to do that in the future. We bleed our patients for blood chemistry at the same time. They come at eight o'clock without their breakfast, and they get through in time so that they can get their breakfast. That gives us very clear serum, if nothing else. I have no data to prove the point as to the statement of some serologists, and a few experiences of mine, that false positives might have occurred from the blood being loaded with the recently absorbed products of digestion. With our own antigen, we also felt occasionally that we did get false positives in miliary TB., or in carcinoma cases. We have not noticed this, however, at all in the short time we have worked with the Kolmer antigen.

There is one other thing that I would like to put into this discussion, if I might, although it is not quite germane to the subject, and that is the anticomplementary serum, not

due to contamination from bacteria, or due to age, but the fresh serum that is anticomplementary. We have tried titrating out the control tubes, that is, putting in the same varying quantities, as we do with Kolmer's quantities, in the tubes without antigen, and we still find that this anticomplementary substance will fix complement in a high degree of dilution. This occurs in patients that we know are syphilitic antibody, and we have felt when we got a serum of this kind it meant syphilis. Of course, it is not ''according to Hoyle.'' Our controls did not clear up, and I would like to know some of your opinions about anticomplementary serum.

Doctor Ives.—I would like to discuss the paper briefly. I believe that we may infer that Kolmer believes that there is an inherent defect in the ordinary cholesterinized antigen. He believes that the ordinary antigen gives nonspecific positive reactions in certain conditions. Such reactions may be interpreted in another way. To me the all-important point is not the kind of antigen used; of equal importance is the proper use of the antigen and of other reagents of the test. I have no reason to believe from my work that cholesterinized human heart antigen gives false positives excepting in very rare instances.

In regard to the matter of anticomplementary sera: Sera which are sufficiently anticomplementary to prevent a report are most rare. However, with positive sera there is often an inhibition in the rate of hemolysis when compared with negative sera.

Doctor Ruediger.—Now, with regard to the positive Wassermanns in the various diseases, I have had the opportunity of testing 150 lepers with a uniformly negative result in all excepting the syphilitic lepers. At the same time, there was a medical officer from the navy, running a parallel series of 150, and he got negative results in all but the syphilitic lepers. About the same time a medical officer of the army was running another series of lepers, and he got 75 per cent positives. The army officer's paper went as far as the Board of Censors, and disappeared. The reason for these positive results was very clear. He used an alcoholic extract of dog heart muscle, and those of you who have tried dog heart muscle, or guinea pig heart muscle, probably got many false positives; at least, I did, as well as others that I have seen working with it. The heart muscle of a dog, or the heart muscle of a guinea pig is not suitable for the preparation of an antigen. I had many cases of frambesia, and they all gave as strongly positive results, as the syphilis does. I tried all the diseases that I could obtain and the only one I did not get was human trypanosomiasis. I have not tested out any of that. Now, as regards the question of anticomplementary serum; I have not yet found an uncomplementary serum that I could not test. Sometimes the anticomplementary property completely disappeared on heating, and if that did not do it, I reheated it at intervals of two or three days, and by the third heating usually it completely disappeared; or one can do it easily by simply running a control tube for every antigen tube, using the human serum in decreasing quantities, because in such cases the specific fixation will always go much further than the anticomplementary property can be detected.

Major Falisi.—I am afraid that the previous speaker (Dr. Ruediger) has left an incorrect impression concerning the Wassermanns as performed in the army.

The official technic is completely described in Colonel Craig's work, with which most of you are familiar, and needs no apology.

Another point upon which I wish to touch is the question of chyle in serum. During my experience at a recruiting depot where things were moving rapidly, it was necessary to take bloods about one and one-half hours after the noon meal and many of these bloods showed chyle, which we feared would interfere with the reaction.

As a matter of fact, the anticomplementary and positive reactions were no more numerous than with the clear serums. Every positive reaction was investigated and only rarely was it impossible to elicit a history

Doctor Kolmer (Closing.)—In reference to Sanford's discussion on the influence of digestion upon serum collected soon after eating, it is my impression that these sera may be at times slightly uncomplementary, but I agree with the last speaker that in Philadelphia we pay little or no attention to this condition, and that the results are uniformly

satisfactory, even with sera which are markedly chylous in appearance. I doubt, however, whether we correctly understood Sanford's reference to anticomplementary sera. I happen to have the advantage of understanding completely, because he was good enough to correspond with me about the matter. Sanford has in the last year or two met with sera from one or two individuals where even fresh sera were anticomplementary. We have met with the same condition twice in the last few days. I believe that it might be related to certain tissue changes that are produced by the administration of the arsphenamines, but it is a subject that requires very careful investigation. Our experience has been similar to that of Sanford, in that these sera have come from cases under treatment. I can assure Ives that no one entertains a higher opinion of cholesterinized antigen than myself. On the other hand, I believe that the ordinary saturated extract of heart muscle that contains approximately four-tenths of one per cent of cholesterol is capable of yielding more nonspecific reactions than the plain alcohol extract. It depends, just as Ives has correctly stated, upon the technical conditions. If the serologist will refuse to accept any cholesterinized extract in which the dose is more than one-fourth or one-fifth of the anticomplementary dose, in other words, if the dose employed is not more than one-tenth of the uncomplementary unit, I agree with him thoroughly that the danger of nonspecific reaction is almost negligible; but, on the other hand, if one employs an extract of this kind in which the dose is about one-half or one-third of the anticomplementary unit, it is my conviction that it increases the danger of nonspecific reactions. In my new antigen I employ cholesterol; but if we refer to the original work of Sachs, we will find that he advocated not more than one-tenth or two-tenths per cent and not the four-tenths that Swift advocated in this country. It has been our experience if we add one or two-tenths per cent cholesterol to an alcoholic extract, that we get all the virtues of cholesterol without increasing the risk of increasing its nonspecific reactions; so we use cholesterol in this amount in my new antigen. There can be no warmer advocate of this practice than myself, but only in two-tenths of one per cent rather than four-tenths. I thoroughly agree with Ruediger about alcohol extracts of guinea pig heart. Our experience has been similar to his own in that these extracts are frequently highly anticomplementary and inferior to extracts of human or beef heart muscle.

Dr. H. E. Robertson, Rochester, Minn., read a paper entitled **Better Postmortem Service.** (For original article see page 486.)

DISCUSSION

Doctor Kolmer.—I, of course, with all here present, feel that every point brought up in this remarkable paper is worthy of discussion; but there is one point in particular that I think is worthy of more than usual attention, and that is the attitude of the undertaker. In Philadelphia, we lose, in the hospitals of the Graduate School of Medicine, a large number of autopsies by reason of the fact that the undertaker gets in touch with the people first, and convinces them that if the doctors perform an autopsy it is not possible for him to guarantee the proper preservation of the body. With the better class of undertakers, we have no trouble at all, because with the care mentioned by Robertson it is easily possible for us to leave the body in such a shape that a good undertaker can preserve it perfectly for burial. The idea, however, occurred to me during the past year that it may be well for us to investigate the kinds of embalming fluids that were being employed to see if we could employ an embalming fluid which the undertaker could first use for the embalming of the body, to be followed by the autopsy. Now then, an embalming fluid of this kind must not produce any gross changes in the organs, nor should it produce histologic changes. In an investigation of the embalming fluids being employed in Philadelphia, we found that all of them were so loaded with formaldehyde that it was impossible to use them for embalming, preliminary to the necropsy, by reason of the profound changes produced in the color and consistency of the organs; but I would like to ask Robertson whether he knows of any fluid that can be used by the undertaker before the body is necropsied? In Philadelphia,

I have been using a fluid that was devised by Hewson, our professor of anatomy in the Graduate School of Medicine, which he employs for the preservation of bodies for anatomic purposes. This fluid preserves the body indefinitely. It contains only one and one-half per cent of formaldehyde, and I now have my autopsy room equipped with all the apparatus which the undertaker requires, and also with this fluid, and whenever I have objections raised by an undertaker, or with the family with whom I am dealing, I simply tell the undertaker to please come in and I will give him all the facilities that he can possibly wish for, including the preserving fluid, and that he is to proceed with the embalming of the body just as if no necropsy were to be done. The only objection to this fluid that has developed in practical experience is the fact that the percentage of formalin is so low that it does not fix the muscles of the face, and after a day or two the jaw begins to drop, so I ask the undertakers to use their own fluid for injecting the head. Dogs embalmed with this fluid have been kept in the basement of the laboratories in close proximity to steam pipes for a matter of three months until they became mummified with absolutely no odor or decomposition. Our experiments are still under way, but we hope that others will take up this matter in a spirit of friendly cooperation with undertakers for the purpose of increasing our percentage of necropsies.

Doctor Robertson (closing).—Kolmer mentioned the undertakers more in detail. I am glad he did. We started a school for embalmers at the University of Minnesota. We had a conference in which the undertakers and the doctors presented their sides. I used to take the undertakers down to the morgue and perform postmortems, and then ask an undertaker who I knew was qualified to repair the body to show them how it should be done. Any such conference could be held at any place, and it is of tremendous value. Sometimes these poor fellows do not know how to repair the bodies after examinations have been made. In regard to his point about the fluid, this is liable to happen more with the inexperienced embalmer. He could make a good fluid himself just as well as he could buy it, but he is a good deal like other people who like to buy their "Quaker Oats" in packages, so he buys his stuff in expensive bottles. Now, one of the simplest means of avoiding this trouble is to tell him if he will use the solution, that is one-half the usual strength, he will find that he will wash out the blood more easily, that he will get a more intensive penetration of the capillary tree than he would with his stronger solution, and that he will be more pleased with the result; and then tell him that if for some reason or other the cheeks are not fixed tightly enough, if he injects the usual strength of his embalming fluid in the arteries, the fixation will be good. Embalming does not depend so much on the strength of the fluid as it does on the wide distribution in the capillary tree.

Brown brought up an illustration of the exact point I wish to make, and I do not want to be offensive, because we are dealing with extremely difficult things to talk about frankly, but we have got to be frank with a thing like this. Why is it that this medical student did not want to have a postmortem examination on his father, and why is it that Brown agrees with the medical student about that? Because an autopsy is not conducted under a decent sort of régime; because it is not done with due respect to the dead. If it is not done decently and in such a way that a relative could come in and see it, why of course, it is objectionable. Relatives occasionally say to us, "I will give you permission to do an examination if you will let me come in and see you do it". I say, "Sure, come along, we will be glad to have you, and we carry out the usual examination. If it is done decently, there can be no objection; and no postmortem ought to be done unless it is done decently. In other words, I do not like to have fellows enter the room with their hats on; I disapprove of smoking in the room; of blood dropped on the floor; of disagreeable sights more than are necessary, because I want to treat that individual just as I want my own body to be treated when an examination is made upon me, and such an examination is going to be made if I can persuade any doctor to do it. Now, it is that attitude towards the examination that will properly educate the physician and the medical student, and will change the thought of such a type as Brown mentioned. It will change this aversion toward a postmortem examination.

Doctor Brown.—An interesting resolution relative to postmortem technic was pre-

sented to the Rochester Medical Association at Rochester, New York, by the local undertakers' association recommending that while doing an autopsy the large vessels be ligated low enough to permit the undertaker to embalm the body satisfactorily. Acting on this recommendation all pathologists in Rochester are carefully tying off all the large vessels before severing from the aorta.

There is one point which was brought up by Robertson on the sentimental side of relatives of deceased persons that has another side as I see it. A medical student who goes through his four years in a recognized medical school is able to visualize the entire picture of a postmortem examination, and it so happens in his case that in the event of the death of one of his relatives he is able to picture to himself the complete postmortem examination in all details as the request is made for permission. If the examination is made he is really subjected to double grief and it seems to me it is rather hard on some types of trained medical men to ask them to suffer twofold unless the case warrants it. Now, the layman does not have to go through quite the same experience; he has in his mind no picture of what is to take place and he suffers from the death of the relative alone.

A friend of mine told me of an autopsy carried out in an undertaking room several years ago that shows how a postmortem examination may react on a relative even after he has given his consent to the same. The day was warm and the door of the examining room was left open though unfortunately it was not guarded. The relative paced back and forth on the walk and finally walked around to the back of the building and casually looking in through the door, saw the operator at work on the autopsy that to him was most gruesome. The shriek that came from his lips was that of extreme agony and pain and one that none of us would care to hear. Here of course, the whole plan was wrong but it brings to our attention the need of the greatest care in the conduct of our postmortem studies.

I think we have got to be careful and tactful, considering the general conditions and obtain permission on first, the cases dying of causes unknown; second, on the doubtful or partly obscure cases and lastly on the routine cases with clean cut diagnosis of the causes of death.

It seems to me that tact is most worth while in obtaining postmortem examinations and the impression should be definitely left with the relatives of the deceased whenever it is possible to do so that a favor is being conferred upon them by making the examination and that it is not being made to satisfy the curiosity of a few physicians and students. The body in the postmortem room should be treated with the greatest consideration; first as a mark of respect to the dead who was beloved by some one; second, that the embalmer may have a chance to restore the appearance so that no observer can see the lines of incision.

The great purpose of medicine is to prevent and relieve human suffering and it seems to me that the suggested wholesale postmortem examinations would be contrary to the fulfillment of this aim and many needless routine autopsies would be made under this policy that would on completion have added not one item of interest, knowledge or satisfaction to anyone except perhaps the statistician.

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These symptoms could not be ameliorated by clearing out the bowels, and after the death of the animal they were found to be associated with an intense fatty infiltration of the liver, unaccompanied by serious lesions in the kidneys—except in one of the four cases—or by any significant histologic change in the voluntary or cardiac musculature. It was concluded that the pancreas must be essential to the physiologic well-being of the animal, quite apart from its rôle in producing insulin. Two reasons were suggested to explain how the absence of the pancreas might cause the fatty changes in the liver; the one being that owing to the absence of pancreatic juice a faulty digestive process would be set up in the intestine, and as a result toxic amines, or similar substances, by their absorption into the portal blood, would injure the liver cells and so lead to fatty changes; and the other, that some pancreatic hormone (other than insulin) necessary for the metabolism of fat had been withdrawn from the organism. In support of the former view it was observed that about fifty per cent of ingested protein reappeared in the feces, often in a completely undigested state and that only a small percentage of fat was absorbed. To this extent the observations lent support to those of Lombroso who, in 1910, concluded that the presence of the pancreas in the body is essential for proper absorption from the intestine, even when it does not actually secrete any digestive juices—a conclusion, however, which had been denied by McClure, Vincent and Pratt.

To put these suggestions as to the cause of the symptoms to the test of experiment the next step was to administer to depancreatized animals not only insulin but the digestive enzymes of the pancreas as well. This has been done by feeding raw pancreas to two dogs, with the significant result that one of them has now survived the pancreatectomy for over fifteen months and the other, for over eight months, both being at the present time in excellent nutritive condition. The daily diet in both cases has consisted of from 200 to 400 grams of meat and fifty grams of raw beef pancreas with from 50 to 100 grams of cane sugar, the dose of insulin, given twice daily, being varied in each case between fifteen and twenty clinical units. There is no doubt that by giving the raw pancreas, the absorption of both protein and fat has been very greatly improved and the absence of any symptoms of hepatic insufficiency may possibly be explained by the fact that no toxic products, due to a faulty (microbal) type of protein digestion, are being absorbed into the blood of the portal circulation. On the other hand, this result does not negative the second suggestion, for it may be that by feeding raw pancreas some hormone, other than insulin, which is not destroyed during the digestive process, is absorbed from the intestine into the blood. To test this possibility, we are at present giving depancreatized dogs trypsin in place of raw pancreas, but the results are not sufficiently forward to report at the present time.

We have attempted to investigate the problem in another way, namely by measuring the amount of esterase in the blood. By use of the stalagmometer method of Rona, we have found in depancreatized animals, that instead of being decreased, this blood-enzyme is decidedly increased in amount. But

it must be remembered that there is probably both an esterase and a lipase in blood and before we consider this part of our work as complete, the blood lipase instead of the esterase must be measured.

Several other facts of possible clinical interest have come to light as a result of these observations. For example, it has been found that the fasting blood sugar is usually about 0.300 per cent, indicating that the animals are more or less constantly hyperglycemic and yet, apparently, without any untoward effects. This does not imply that it would be safe to allow a human diabetic to carry so high a blood sugar, for there is an essential difference in the clinical condition as it exists in him and in the depancreatized dog; namely, that the diabetic patient still retains some islet tissue which must be protected from overstrain by keeping the blood sugar as low as possible. What the observation does show is that increased blood sugar per se does not cause the degenerative changes which some clinicians have imagined it to do.

Another fact of interest is that one of the dogs (9 months) has gone through a normal pregnancy and is now bringing up two of her family of five. She went through the pregnancy without any perceptible change in the sugar balance but a few hours after delivery she developed severe symptoms of hypoglycemia from which, however, she spontaneously recovered. It would appear that the drawing of sugar from the body through the milk was responsible for these symptoms. In view of the belief, due originally to experiments by Carlson and Drennan, that the developing fetuses may secrete sufficient insulin to protect the mother against the effects of pancreatectomy, the foregoing observation is of great interest. It does not, however, entirely disprove this hypothesis for, although in our experiments no perceptible change occurred in the carbohydrate balance during the pregnancy, it may be that this was because so much insulin was being given in any case that the small contribution of the fetuses could not make itself felt. Since the glucose equivalent of insulin is so very much greater with small as compared with large doses (Allan), the fetal contribution of this hormone, in the absence of injections of the hormone, might have a pronounced beneficial effect on the mother.

Finally, it should be pointed out that there is every indication that these two animals are completely depancreatized, for both of them become intensely diabetic whenever the injections of insulin are discontinued. It is very significant also that in three days and over, after withdrawal of insulin and food from other similarly treated animals the blood sugar has been found to be decidedly higher in fat as compared with thin animals. The question arises whether this may indicate the derivation of sugar from fat (Chaikoff, Markowitz and Simpson).

Another problem of clinical interest concerns the source of insulin in the animal body. The isolation of substances capable of lowering the blood sugar of normal animals from all sorts of materials, both animal and vegetable in origin (Collip, Winter and Smith, Best, etc.), and even from the tissues of diabetic animals (Best and Scott), naturally raises the question as to whether the isles of Langerhans of the pancreas can, after all, be the

exclusive source from which the intact animal derives this hormone. On account of these facts some authors, notably Oertel and Swale Vincent have formally challenged the islet hypothesis of diabetes. Supplementing the excellent anatomic and pathologic evidence upon which originally this hypothesis was built, three proofs of a different character arising out of the present work may be added. The first of these is the fact, alluded to above, that the discontinuance of the injection of insulin into depancreatized dogs causes severe symptoms of diabetes to supervene within a day or so, followed by death in a remarkably short period of time unless insulin is again administered. If tissues other than the pancreas can produce insulin why do they fail to do so under these conditions? The insulin that is extractable from them must be incapable of functioning in the depancreatized animal and pancreatic insulin only can prevent diabetes.

The second and third proofs have been obtained by work on certain of the bony fishes (Teleostei) such as the angler fish (Lophius piscatorius) and the sculpin (Myoxocephalus) in which the pancreas does not contain the usual islets of Langerhans, these being collected into encapsulated glands of considerable size, situated outside the pancreas, usually in the neighborhood of the spleen and the pylorus. Two years ago, I found that extracts prepared in the usual way from these principal islets, as they are called, contained very large amounts of insulin whereas none was obtainable by exactly the same technic from the pancreas itself. McCormick and Noble have also prepared large amounts of insulin from the principal islets of the cod and pollock. More recently, Swale Vincent, Dodds and Dickens have found that *some* insulin may be prepared by the picric acid process of Dudley from the pancreas of these fishes but they admit that the yields obtainable are very small compared with those from the islets. It may be that the few scattered microscopic islets, observed by Slater Jackson and by Scott in the pancreas itself are responsible for these effects. But even this overwhelming presence of insulin in the principal islets as compared with the pancreas proper is not absolute proof of the insular hypothesis, and since we may consider the present as a test case, not only of the islet hypothesis, but also of the general principle that difference in function is often related to essential differences in structure, McCormick and I have attacked the problem in another way. In the sculpin it is possible, by performing laparotomy, to excise the principal islets without much trouble. The operation lasts from fifteen to twenty minutes and the fish soon recovers from its immediate effects and swims about in a normal manner when returned to sea water. The blood sugar measured in fish removed on various days following the isletectomy was found to be markedly raised, indicating the existence of severe diabetes but before attributing this to the isletectomy it was necessary to take into account the fact that for some days (four to five) after exposure to air the blood sugar—normally about 0.035 per cent in this fish—is apt to be raised (asphyxial hyperglycemia). We found, however, that it was decidedly higher in fish from which the islets had been removed and more decisive still, that the hyperglycemia continued after the fifth day, by which time that due to

asphyxia had almost invariably disappeared in unoperated fish. There can be no doubt that removal of the principal islets without disturbance of the pancreas causes marked hyperglycemia in the sculpin and since this is the cardinal symptom of diabetes it seems justifiable to conclude that the fish has become diabetic. In support of this it may be mentioned that the liver of the diabetic fish was found to contain distinctly more fat and less glycogen than those of the controls.

The final proof is still lacking, namely to see whether administration of insulin to the isletectomized fish would prevent the development of the diabetic symptoms. This experiment we hope to do at the earliest opportunity. Meanwhile, however, we have investigated the effect of insulin on normal fish (sculpin) or on fish rendered hyperglycemic by asphyxia and in neither case have we been able to show that it has any very marked influence in lowering the blood sugar. This is strange and may be related to the low body temperature which allows the excess of injected insulin to be excreted before it can act or may be due to a mobilization of sugar out of glycogen or other sources which proceeds at the same rate as insulin can act.

—J. J. R. M.

Variation of Virulence in Tubercle Bacilli

IF we lacked evidence that tubercle bacilli vary in virulence we should be sure that our observation was at fault, since it is certain that all living organisms vary in all their attributes.

As a matter of fact the evidence is plentiful. The characteristic differences which the human, bovine, and avian types show in their power to produce disease in several animal species are well known. Again these characteristic differences are known to be variable, inconstant in degree.

The greatest volume of evidence on this point relates to the "human" type or group. The members of this group are characterized in a general way by slow but plentiful dry growth on media containing glycerin, and by relatively low virulence for cattle, rabbits, and birds. Most, but not all of them possess rapidly lethal virulence for the guinea pig, the animal most used in studying them.

As a rule, strains of this type kill guinea pigs in about two to three months after subcutaneous inoculation. Within wide limits the number of bacilli injected seems to have little effect on the duration of life,[1] though very small numbers of bacilli from virulent strains may cause a comparatively chronic disease.[2, 3] But a number of strains have been reported which even in large doses caused death only after many months or not at all.

The Saranac Lake R1 strain, one of the best known human strains of low virulence, was recovered by Trudeau in 1891 from a case of general miliary tuberculosis. At first it showed standard virulence for guinea pigs, but after two or three years its virulence declined greatly, and now for some thirty years all cultures made from it have shown very low pathogenicity. It has

been extensively studied by Trudeau,[4] Krause,[5] and Baldwin and Gardner.[6] In the great majority of instances it produces in guinea pigs a very benign glandular tuberculosis which shows a marked tendency to heal, and does not interfere with the general well-being of the animal or shorten its life. However, this single strain exhibits variation in virulence, for Baldwin and Gardner observed fatal, though chronic, infections after both inhalation and subcutaneous inoculation, and Rosenberger[7] after intraperitoneal. We have observed a fatal case of fifteen months' duration after subcutaneous injection. Inoculation with tissues and cultures from this animal caused a disease more rapidly fatal than the original R1 infection, but in some cases very much less so than the usual experimental infection.

Numerous other more or less attenuated strains have been reported by Jousset,[8] Arloing,[9] Raw,[10] and others. Dreyer[11] has worked with a strain cultivated by Schottelius, which usually killed guinea pigs in four to ten (commonly six to eight) months. We have reported the production of chronic tuberculosis in guinea pigs by implantation of incubated tuberculous tissues.[3] From such a case a strain has been cultivated which exhibits rather low virulence, though again the disease produced is less chronic than that in the original animal. This strain differs in its history from other avirulent strains, most of which have resulted from prolonged cultivation. We have produced chronic guinea pig tuberculosis by inoculation with dry cultures—a well-known possibility—and also by injecting sputum from a patient with an old fibrous pulmonary tuberculosis in which the bacilli are constantly very long and beaded. Studies of the varying virulence of different strains and of different cultures of the same strain are being continued by Boissevain and Ryder in the laboratories of the Colorado Foundation for Research in Tuberculosis.

The whole question of loss of virulence and possible recovery of virulence has assumed vital importance owing to Calmette's recent revival of the subject of protective vaccination with apparently avirulent strains of tubercle bacilli.[12, 13, 14, 15] Calmette reports that after prolonged cultivation on bile-potato-glycerin media the bacilli lose all power to cause disease when inoculated intravenously or by any other route commonly employed, but remain alive in the body for a year or so, and that during this time the body is refractory to virulent infection, provided it has never been infected before. The behavior of the R1 strain leads one to feel that there may be considerable risk in this procedure.

REFERENCES

[1] Cobbett: The Causes of Tuberculosis, 1917.
[2] Gilbert and Gregg: Amer. Assn. of Immunol., 1915, Med. Rec., New York, July 24-31, 1915.
[3] Webb, Ryder, and Gilbert: Trans. Nat. Tuberc. Assn., 1923, p. 290.
[4] Trudeau: Brit. Med. Jour., 1897, ii, 1849.
[5] Krause: Am. Rev. Tuberc., March, 1919, iii, 1.
[6] Baldwin and Gardner: Ibid, August, 1921, v, 429.
[7] Rosenberger: Jour. Lab. and Clin. Med., November, 1924, x, 140.
[8] Jousset: Cong. internat. de la tuberc., Par., 1905, i, 854.
[9] Arloing: Acad. des Sci., June 18, 1906, Nov. 27, 1909.
[10] Raw: Brit. Med. Jour., April 23, 1921, 594.

[11]Dreyer: Brit. Jour. Exper. Path., 1923, iv, 146.
[12]Calmette: L'Infection bacillaire et la Tuberculose, 1920.
[13]Calmette, Boquet, and Nègre: Ann. de l'Inst. Pasteur, September, 1921, xxxv, 561.
[14]Calmette: Brit. Jour. Tuberc., July, 1921, xv, 103.
[15]Calmette: Commun. à l'Acad. de Méd., Paris corresp., Jour. Am. Med. Assn., August 9, 1924, 453.

—*G. B. W. (C. T. R.)*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Basal Metabolism in Health and Disease**

BY far the most comprehensive and generally useful single volume on this subject which the reviewer has as yet encountered. The work begins with a brief historical sketch followed by a summary of the salient points of the metabolism of carbohydrate, fat and protein. Those laws of physics which have a bearing upon the performance of metabolism tests are briefly recapitulated. Internal and external respiration are described in some detail. There next follows an enumeration of general principles governing the determination of basal metabolic rates with detailed description of the various instruments in clinical use, and a discussion of their relative values. The normal basal metabolism receives detailed consideration, while nearly 200 pages are devoted to metabolic alterations in disease. Of especial importance and interest aside from thyroid disease is the author's description of the metabolism in diabetes mellitus.

General Cytology†

THE Marine Biological Laboratory at Woods Hole, Mass., is known to all students of biology especially as a place where one may study the relatively simple forms of marine life so that we may learn more of the fundamental biologic laws of nature. Investigators from many parts of the country customarily spend their summers at Woods Hole, where with thoroughly adequate facilities each studies that particular biologic phenomenon which has for him the greatest interest and presents the most alluring problem.

In 1922 a group of these individual investigators initiated collaborative studies with a view to producing a compilation of monographs dealing with

the life processes of the living cell. The result is a 750 page volume entitled General Cytology. The individual writers have incorporated their own work into a general critical view of the literature so that each subject has been covered in every detail.

In the volume one may find most of what is known today regarding the living cell, including its physics, its chemistry, both general, colloidal and biochemistry, methods of fertilization, growth and cellular differentiation, with comprehensive discussions of the theories of heredity as applied to the individual cell.

The chief value of this work will be as an authoritative handbook for students of biology, both general and medical, and as a reference volume for all whose interest is in the general biologic sciences.

Basal Metabolism*

A HANDY reference manual written especially for those interested in the clinical aspects of basal metabolic rate determination. The principles of direct and indirect calorimetry are discussed. The physiology of heat production and heat regulation is presented in considerable detail and the effect of certain drugs thereon is briefly summarized. Chapters are devoted to study of the metabolism in hyper- and hypothyroidism and in various other diseases. The appendix contains tables and charts, helpful time-savers in the performance of metabolism tests.

The Examination of Patients†

A THOROUGHLY valuable handbook for those who desire to perform exhaustive physical examinations. Much additional space might well have been given to the recording of the anamnesis and the various subjective symptoms, for this indeed must be reckoned a part of the examination. Again, we should have liked to have seen more detailed discussions of the various special examinations, such as pelvic examination, proctoscopic examination, examination of the visual fields and other similar procedures which the internist often through a feeling of inexperience refers to some other specialist without having first undertaken himself.

These are the special fields in which the internist feels a need for a sufficiently detailed reference work applied particularly to his own requirements. It has been very well covered in the author's description of the neurologic examination.

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*Basal Metabolism. By John T King, Jr. Cloth. Pp. 118 Price $2.50. Williams & Wilkins Co., Baltimore, 1924.

†The Examination of Patients. By Nellie B. Foster, M D, Associate Physician to the New York Hospital; Associate Professor of Medicine at Cornell University, College of Medicine. Pp. 253. Illustrated. Cloth. Price $3 50. W. B. Saunders Co, Philadelphia, 1924.

The American Society of Clinical Pathologists

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The Next Annual Meeting Will Be Held in Philadelphia, May 21-23, 1925
Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 21, 22 and 23, 1925, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering next year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.

CLINICAL AND EXPERIMENTAL

A STUDY OF THE GRAM-POSITIVE COCCI PRESENT IN THE VAGINA AND CERVICAL CANAL OF THE UTERUS*

By Lloyd Arnold, M.D., and Louis Brody, M.D., Chicago

THE gynecologists recognize the Gram-positive cocci as important constituents of the bacterial flora of the vagina and the cervical canal.[1] These cocci are almost always present in cultures obtained from this part of the female genital tract. We were interested in the question of the relation between various cocci present in vaginal and cervical regions and also between those of the vaginal and fecal cocci. We have used certain cultural and biologic characteristics to differentiate the groups, such as sugar fermentations, limiting hydrogen-ion concentration and pathogenicity for mice.

TECHNIC

Cultural media were the same as those already described.[2] The methods for the determination of the limiting hydrogen-ion concentration[3] and pathogenicity for mice[4] were the same as we have previously employed. The swabs containing the material for bacteriologic examination were obtained in the following manner: After a speculum was inserted into the vagina, swabs were obtained from the sides of the vagina between the blades of the speculum at the respective levels. The cervix was cleaned with sterile absorbent cotton by repeated application, then a swab was inserted in the cervical canal. All swabs were put in dry sterile test tubes and used to seed the respective media within three hours after being obtained. Our material was taken from patients in the out-door dispensary of Mercy Hospital; all had a leucorrheal discharge and pathologic lesions associated with such discharge.

OBSERVATIONS

The appearance of colonies of staphylococcus was noted. These were typical colonies; only a few hemolytic varieties were encountered. The blood agar plates were examined by transmitted light and distinct greenish areas were observed around the (streptococcus) colonies. Some colonies appearing as the "indifferent" variety would upon subculture produce some methemoglobin. Subcultured into vitamine bouillon,[2] the staphylococcus always produced a uniform cloudiness, and sedimentation became apparent after incubations of eighteen to twenty-four hours. The streptococcus in this medium varied from a uniform cloudiness to a typical granular sediment adhering to the sides and settling to the bottom of the tube; the bouillon remained perfectly clear. All gradations between these two extremes were observed with Gram-positive cocci that grew in chains. Morphologic studies were always made upon smears taken from plain vitamine bouillon (P$_H$ 7.6-7.8) after eighteen to twenty-four hours' incubation.

We found many colonies on blood agar that were small (1 mm. or less) granular, lusterless, firm, grayish, (streptococcus) with a dark brownish color noticeable around the colony on transmitted light. These colonies were smaller than the streptococcus colonies and the color was not observed unless plates were carefully studied against a strong light. When colonies of this type were subcultured to bouillon, the morphology of a smear from a twenty-four-hour-old colony was that of single and diplococcus. Chains were never formed; at times four members in diplo-arrangement would be adherent, or the same number in tetrad-arrangement. Subcultured for several days upon bouillon, with intermittent blood agar plate transfers, the morphology of these cocci was not changed. The growth in the bouillon (P$_H$ 7.6-7.8) was nearer the streptococcus than the staphylococcus type of growth. The sediment was seldom granular, and usually showed slight viscid, stringy shreds when agitated; sedimentation was mostly collected on the sides of the tube and only settled to the bottom after agitation. This type of coccus has probably been considered as a streptococcus by other workers. We have found the morphology on smears from a twenty-four-hour-old bouillon culture to be different from both the streptococcus and the staphylococcus. Certain cultural characteristics help to differentiate it from the streptococcus, but it resembles the streptococcus much more than it does the staphylococcus. This coccus resembles the enterococcus described by Thiercelin[5] and recent investigations by Dible[6] seem to differentiate an enterococcus from the streptococcus fecalis group of Andrews and Horder.[7]

We have not investigated this coccus sufficiently to warrant our calling it an enterococcus in the sense implied by Weissenbach[8] and other French investigators. But we feel that if the cocci of this group were classed as streptococci, our incidence of streptococci would be too high and we have found the characteristic previously mentioned to be constant for this group of cocci. We will refer to this cocci as the enterococcus in this paper, meaning by that term a Gram-positive coccus; the morphology on smears from a twenty-four-hour bouillon culture shows a single and diplococcus, with isolated cocci in

chains of four members or in groups of four members. On blood agar plates the colony is small (1 mm. or less), gray, granular, lusterless, with a brownish discoloration of the environment when examined by transmitted light.

We have used lactose, mannite and salicin as fermentable substances in previous work with streptococci. In this work we have used the same substances for purposes of comparison. The number of fermentable substances must for technical reasons be limited in most bacteriologic work in connection with clinical material.

EXPERIMENTS

One hundred seventy strains of Gram-positive cocci were isolated from the vaginal tract, forty-eight staphylococcus, sixty-six enterococcus and fifty-six streptococcus. Two hundred sixty-two strains were isolated from the cervical canal of the uterus, seventy staphylococcus, ninety-six enterococcus and ninety-six streptococcus. Table I shows the reactions of these strains according to their ability to utilize lactose, mannite and salicin as carbon sources of energy.

Mannite fermentation has been found to be rather constant for most fecal strains of cocci, especially the streptococci.[7] In Table II, the mannite fermentations of vaginal and cervical strains of streptococcus are compared with those isolated by Oppenheim from the feces and by Arnold from the pharynx. It will be noticed that the vaginal strains are nearer the fecal strains in their

TABLE I

| | | VAGINAL TRACT | | | | CERVICAL CANAL | | | |
| | | STAPHYL-OCOCCUS | ENTERO-COCCUS | STREPTOCOCCUS | | STAPHYL-OCOCCUS | ENTERO-COCCUS | STREPTOCOCCUS | |
				NON-HEMOL.	HEMOL.			NON-HEMOL.	HEMOL.
Lactose	+								
Mannite	+	14	22	14		6	12	14	4
Salicin	+								
Lactose	+								
Mannite	+	2	2	2		4	6	5	
Salicin	−								
Lactose	+								
Mannite	−	10	6	10		8	18	10	4
Salicin	−								
Lactose	−								
Mannite	+	6	10	6	2	4	8	8	4
Salicin	+								
Lactose	−								
Mannite	−	2	16	2		10	20	6	
Salicin	+								
Lactose	+								
Mannite	−	6	4	8		18	12	20	
Salicin	+								
Lactose	−								
Mannite	+	2	0	0		2	6	0	
Salicin	−								
Lactose	−								
Mannite	−	6	6	12		18	14	21	
Salicin	−								
Total		48	66	54	2	70	96	84	12

TABLE II

STREPTOCOCCUS

FERMENTABLE SUBSTANCE	VAGINA		CERVIX		FECES X		PHARYNX =	
	NUMBER STRAINS	PER CENT	NUMBER STRAINS	PER CENT	NUMBER STRAINS	PER CENT	NUMBER STRAINS	PER CENT
Mannite positive	24	42.86	35	36.46	239	.76.0	24	14.0
Mannite negative	32	57.14	16	63.54	84	24.0	150	86.0

x Oppenheim[9]
= Arnold[2]

TABLE III

MANNITE FERMENTATION

	NEGATIVE		POSITIVE	
	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT
Staphylococcus				
Vagina	24	50.0	24	50.0
Cervix	54	77.15	16	22.85
Enterococcus				
Vagina	32	48.5	34	51.5
Cervix	64	66.66	32	33.33
Streptococcus				
Vagina	32	57.14	24	42.86
Cervix	61	63.54	35	36.46

TABLE IV

	VAGINA				CERVIX			
	NON-PATHOGENIC		PATHOGENIC		NON-PATHOGENIC		PATHOGENIC	
	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT	NO. STRAINS	PER CENT
Staphylococcus	10	83.4	2	16.6	6	75.0	2	25.0
Streptococcus	24	75.0	8	25.	16	61.5	10	38.5
Enterococcus	9	75.0	3	25.	17	60.8	11	39.2

mannite fermenting power than are the cervical strains; the pharyngeal strains are merely given to show the other extreme in mannite fermentation.

In Table III, the mannite fermentations of the vaginal and cervical strains of staphylococcus, the so-called enterococcus and the streptococcus, are compared. Here it will be noticed that all of the vaginal flora of the Gram-positive coccus group ferment mannite more than those of the corresponding cervical flora. The limiting hydrogen-ion concentration for the three groups of Gram-positive cocci studied, did not differ markedly from one another. They varied from P_H 4.5 to P_H 5.6. The vaginal flora (170 strains in all) did not differ markedly from the cervical flora (262 strains) in this respect. The average H-ion concentration that limited growth was P_H 5.0. Table IV gives the results of the pathogenicity experiments with mice.

DISCUSSION

Avery and Cullen,[10] Brown[11] and Arnold[3] and others have divided the streptococci into human and bovine types by the limiting H-ion concentration. The human type is more sensitive to an acid environment and ceases to grow

at a H-ion concentration of P_H 4.8 to 5.4. The bovine type, on the other hand, is not so sensitive and the limiting H-ion concentration necessary to inhibit growth was found to vary from P_H 4.3 to 4.5. Brody and Arnold[12] have shown that there is no relationship between the limiting H-ion concentration of streptococci and their pathogenicity for mice. We suggested that the difference between the so-called human and bovine types was merely one of environmental conditions of growth. Streptococci isolated from areas in contact with an inflammatory exudation were of the human type; the bovine types came from areas not associated with exudation.

This suggestion is further confirmed as a result of the experiments reported in this paper. The total Gram-positive cocci isolated from the vaginal tract was 170, from the cervical 262. The average limiting H-ion concentration was P_H 5.0, the variations were between P_H 4.7 and 5.6. In other words, they were all within the human or pathogenic range. This we think was a result of the contact of these strains with the exudation present in the cervix and vagina of the cases studied. The bacteria found in the cervical canal are an extension upwards of those in the vaginal tract. The vaginal bacterial flora resemble in many respects certain fecal types. The Gram-positive cocci of the cervical canal differ from those found in the vaginal tract. There is a gradual loss of the power to ferment mannite, so typical of strains of fecal origin, and they become more pathogenic for mice. Both of these differences are probably due to the more parasitic mode of existence of the microorganism in the cervical canal under the condition found in our cases.

We have used only a few strains for our pathogenicity experiments and for this reason we do not place much value upon information gained by studying such a small number of cocci picked at random. We feel that a more detailed study of the pathology, etc., of the clinical cases from which the strains were isolated would be necessary before drawing definite conclusions as to our pathogenicity experiments.

SUMMARY

The Gram-positive cocci of the vaginal tract resemble closely the corresponding cocci of fecal origin. Those cocci present in the cervical canal of the uterus under various pathologic conditions, resemble those of vaginal origin. With gradual extension of the fecal types up through the vagina to the cervix, certain biologic changes take place: the power to ferment mannite is gradually decreased and an increased pathogenicity for mice is probably acquired. The differences in the limiting H-ion concentration of Gram-positive cocci is not an index to their pathogenicity, but those most sensitive to the hydrogen-ion concentration (P_H 4.8 to 5.4) have recently been in contact with an inflammatory exudate.

REFERENCES

[1] Curtis: Jour. Am. Med. Assn., 1923, lxxx, 161.
[2] Arnold: Jour. Lab. and Clin. Med., 1920, v, 587.
[3] Arnold: Ibid., 1921, vi, 312.
[4] Brody and Arnold: Ibid., 1923, viii, 387.
[5] Thiercelin: Compt. rend. Soc de biol., 1899, v, 269.
[6] Dible: Jour. Path. and Bacteriol., 1921, xxiv, 3.

[7]Andrews and Horder: Lancet, 1906, xi, 708.
[8]Weissenbach: Compt. rend. Soc. de biol., 1918, lxxi, 819.
[9]Oppenheim: Jour. Infect. Dis., 1920, xxvi, 117.
[10]Avery and Cullen: Jour. Exper. Med., 1919, xxix, 215.
[11]Brown: Ibid., 1920, xxxi, 35.
[12]Brody and Arnold: Jour. Bacteriol., 1923, viii, 307.

LIVER FUNCTION IN VARIOUS DISEASES AS TESTED BY THE ADMINISTRATION OF LEVULOSE*

By Reuben Finkelstein, M.D., and Max Dannenberg, M.D., Brooklyn, N. Y.

IT is a well-known fact that the liver function is disturbed in many diseases both in acute and chronic conditions. Very often this disturbance in function is due to permanent pathology as is found at autopsy. In most cases the pathology is not severe enough or does not become permanent and the function returns to normal after the disease subsides. After pneumonia or influenza, permanent destruction of liver tissue is seldom found, although during the course of these diseases, its functions may be greatly disturbed.

It is evident that it would be very difficult to test each one of the numerous liver functions. We feel that this may be unnecessary in that disturbance of one function may be sufficient evidence of some degree of disturbance of all functions of the liver. It is hard to imagine that the ureagenic function of the liver will be altered without some change at the same time in its glycogenic function. The cause of these changes evidently is due to the diminished activity of the cells, which will affect all of its functions.

One of the most important functions of the liver is its glycogenic function, the conversion and storage of glycogen.

Since 1875 various investigators have sought to prove disturbed liver function through the ingestion of sugar. In that year Colrat[1] suggested the ingestion of 150 to 200 grams of cane sugar with subsequent examination of the urine. He found glycosuria in many conditions especially neurosis, hyperthyroidism, etc. He attributed this glycosuria to disturbed glycogenic function of the liver. The objection to this test is that saccharose is first converted into glucose in the alimentary tract. The rate of conversion varies in different people thereby introducing an unknown factor.

Later, glucose was used with the idea that no conversion would be necessary. But other organs besides the liver convert and store glycogen. This was proved definitely by Sachs[2] in 1899. He removed the livers of frogs and observed the tolerance to various sugars. The only sugar not used up was levulose. All the other sugars were converted into glycogen, thus proving that other organs besides the liver have the power of conversion and storage of glycogen.

Taking the above experiment into consideration, i.e., that levulose was

TABLE I
CASES IN WHICH LEVULOSE FUNCTION TEST WAS PERFORMED

NO.	NAME	FASTING	½ HOUR	1 HOUR	2 HOURS	DIAGNOSIS
1	M.M.	.080	.083	.083	.078	Fibrosis of Lung
2	M.G.	.084	.120	.140	.106	Bronchial Asthma
3	J.K.	.080	.083	.083	.084	Gastric Ulcer
4	M.S.	.116	.122	.125	.132	Acute Cholecystitis
5	H.G.	.086	.088	.086	.070	Arthritis
6	J.R.	.055	.070	.070	.084	Influenza
7	I.S.	.076	.076	.060	.062	Duodenal Ulcer
8	L.T.	.076	.076	.075	.075	Pneumonia
9	J.R.	.109	.090	.083	.084	Lung Abscess
10	L.K.	.075	.075	.068	.064	Myocarditis
11	F.R.	.092	.100	.125	.150	Arthritis
12	M.R.	.083	.100	.108	.125	Pulmonary Tuberculosis
13	N.O.	.060	.160	.140	.100	Gastroduodenitis with Jaundice
14	J G	.087	.120	.107	.100	Chr. Cholecystitis
15	B.S.	.100	.120	.115	.106	Hydropneumothorax
16	B.K.	.106	.130	.130	.120	Chr. Myocarditis
17	Y.M.	.080	.125	.133	.106	Bronchial Asthma
18	G.A.	.100	.160	.160	.162	Influenza
19	T.M.	.150	.200	.250	.187	Lobar Pneumonia
20	S.R.	.100	.150	.160	137	Myocarditis
21	A.H.	.105	.110	.125	.200	Carcinoma Pancreas
22	P.S.	.138	.145	.166	.200	Chr. Cholecystitis
23	I.G.	.150	.155	.166	.200	Chr. Val. Disease with Ascites
24	R.R.	.125	.150	.194	.143	Cholecystitis
25	A.S.	.080	.080	.082	.084	Myocarditis
26	T.F.	.135	.130	.135	.135	Arteriosclerosis with Nephritis
27	J.F.	.140	.160	.135	.140	Arteriosclerosis
28	F.B.	.110	.100	.110		Chronic Nephritis
29	P.S.	.105	.105	.110		Chr. Appendicitis
30	H.A.	.100	.090	.090		Acute Tonsillitis
31	H.S.	.150	.150	.155		Arteriosclerosis with Nephritis
32	E.D.	.125	.150	.140	.135	Chr. Cholecystitis
33	R.L.	.110	.110	.100		Subacute Appendicitis
34	F.R.	.120	.110	.110		Pelvic Inflammation
35	B J.	.110	.108	.110		Ulcerated Cervix
36	E.F.	.110	.135	.130	.120	Chr. Myocarditis
37	H.L.	.130	.130	.125		Carcinoma Lung
38	F.G.	.140	.135	.135		Arteriosclerosis with Nephritis

the only sugar not converted and stored as glycogen by the liverless frogs, Strauss[3] in 1901, formulated a new test for liver deficiency. He gave 100 grams of levulose in weak tea on a fasting stomach and examined the urine. No food was given after the previous evening meal. At 6 A.M. he obtained a normal urine, then gave the levulose. The urine was examined for levulose at hourly intervals for four hours. He found 76 per cent of negative tests in clinically normal liver cases, and positive in 83 per cent of clinically abnormal liver cases. The presence of a sufficiently large number of positive reactions in normal cases and negative reactions in abnormal cases is final proof that this test is not conclusive.

Many investigators have worked on this test and have given levulose in various quantities, but in all cases only the urine was examined at varying intervals. The great fault was that often too much levulose was given and an alimentary levulosuria resulted. Again the permeability of the kidneys to levulose varies with different individuals. This test also soon fell into disuse because of its uncertainty.

In 1921, McLean and De Wesselow[1] studied blood sugar curves of normal adults and that of diabetics after the ingestion of various sugars, such as glucose, levulose, saccharose, galactose, etc. All these sugars, excepting levulose, provoked a rise in the blood sugar. As little as 25 grams of these sugars provoked a maximal rise. Larger doses were incapable of increasing the level, merely tending to prolong the time during which this level was main-

TABLE II

DISEASES IN WHICH LEVULOSE FUNCTION TEST WAS FOUND POSITIVE

NO.		BLOOD SUGAR FASTING STOMACH	½ HOUR AFTER INGESTION 50 GRAMS LEVULOSE	1 HOUR AFTER INGESTION	2 HOURS AFTER INGESTION
2	Bronchial Asthma	.084	.120	.140	.106
17	Bronchial Asthma	.080	.125	.133	.100
4	Acute Cholecystitis	.116	.122	.125	.132
11	Arthritis	.092	.100	.125	.150
6	Influenza	.055	.070	.070	.084
18	Influenza	.100	.160	.160	.162
19	Pneumonia	.150	.200	.250	.185
20	Myocarditis, Enlarged Liver	.100	.150	.160	.137
36	Myocarditis, Enlarged Liver	.110	.135	.130	.120
16	Myocarditis, Enlarged Liver	.100	.130	.130	.120
12	Pulmonary Tuberculosis	.083	.100	.108	.125
13	Gastroduodenitis with Jaundice	.060	.160	.140	.100
14	Chr. Cholecystitis	.087	.120	.107	.100
22	Chr. Cholecystitis	.138	.145	.166	.200
24	Chr. Cholecystitis	.125	.150	.194	.143
32	Chr. Cholecystitis	.125	.150	.140	.135
15	Hydropneumothorax	.100	.120	.115	.106
21	Carcinoma of Pancreas	.105	.110	.125	.200
23	Chr. Val. Disease with Ascites	.150	.155	.166	.200

TABLE III

DISEASES IN WHICH LEVULOSE LIVER FUNCTION TEST WAS FOUND NEGATIVE

NO.		BLOOD SUGAR FASTING STOMACH	½ HOUR AFTER INGESTION 50 GRAMS LEVULOSE	1 HOUR AFTER INGESTION	2 HOURS AFTER INGESTION
1	Fibrosis Lung	.080	.083	.080	.078
3	Gastroduodenal Ulcer	.080	.083	.083	.084
7	Gastroduodenal Ulcer	.076	.076	.060	.062
5	Arthritis	.086	.088	.086	.070
8	Pneumonia	.076	.076	.075	.075
9	Lung Abscess	.109	.090	.083	.084
25	Myocarditis	.080	.080	.082	.084
10	Myocarditis	.075	.075	.068	.074
26	Arteriosclerosis, Nephritis	.135	.130	.135	.135
27	Arteriosclerosis, Nephritis	.140	.160	.135	.140
31	Arteriosclerosis, Nephritis	.150	.150	.155	.155
38	Arteriosclerosis, Nephritis	.140	.135	.135	
28	Chr. Nephritis	.110	.100	.110	
29	Chr. Appendicitis	.105	.105	.110	
30	Acute Follicular Tonsillitis	.100	.090	.090	
33	Subacute Appendicitis	.110	.110	.100	
34	Pelvic Inflammation	.120	.110	.110	
35	Ulcerated Cervix	.110	.108	.110	
37	Carcinoma Lung	.130	.130	.125	

tained. Levulose on the contrary, had no effect on the blood sugar in persons with normal livers. This proves the original experiment of Sachs in 1899 as mentioned above.

Spence and Brett[5] later, found that the ingestion of 50 grams of levulose will show no rise in the blood sugar. More than 50 grams may cause an alimentary levulosuria because even a normal liver has a limit of storage and all levulose above this limit will be spilt over into the blood and excreted by the kidneys. The rationale of this test therefore depends upon the fact that the normal liver is able to convert and store 50 grams of levulose given at one time. If the liver is in any way damaged the entire amount would not be taken up by its cells and some levulose would appear in the blood.

We have performed the levulose test on thirty-eight consecutive patients admitted to hospital. No food was given after the evening meal. Next morning, the patient still fasting, blood was taken and its sugar content determined. Fifty grams of levulose dissolved in water was administered. The blood sugar was again determined at half hour intervals for two hours. The blood in cases one to twenty-six was tested by the Benedict method; in cases twenty-seven to thirty-eight by the Epstein method. The positive and negative results are about equally divided. The nineteen cases in which no increase in blood sugar was observed represent fourteen different diseases. The other nineteen cases representing twelve various diseases gave an elevated blood sugar curve for a period of at least two hours.

While the series is rather small for conclusive deductions, it will be noted that the test was positive in acute and chronic gall bladder infections, (five cases); in icterus, in carcinoma of the pancreas, and in chronic passive congestion of the liver (four cases).

The curve was also positive in a few other conditions in which there was no other clinical evidence of hepatic injury.

If we assume that the abnormal blood sugar curves observed are indicative of altered liver function, it would appear that such alteration occurs in some cases in which we have, as a rule, not suspected hepatic damage.

REFERENCES

[1]Colrat, mentioned by Barton.
[2]Sachs, H.: Ueber die Bedeutung der Leber für die Verveithung der Verschiedenen Zuckerarten im Organismus; Zeitschr. f. klin. Med., 1899, xxxviii, 87.
[3]Strauss: Ueber den Einfluss der Verschiedenen Zuckerarten auf die Zuckerausscheidung beim Menschen, Berl. klin. Wchnschr., 1898, xxxv, 398.
[4]McLean and De Wesselow: Estimation of Sugar Tolerance, Quart. Jour. Med., 1921, xiv.
[5]Spence and Brett: The Use of Levulose as a Test for Hepatic Inefficiency, Lancet, December, 1921, ii, 1362.

OBSERVATIONS ON THE RELATION OF URINARY P$_H$ TO SALT AND WATER METABOLISM IN A CASE OF NEPHROSIS*

By Milton B. Cohen, M.D., Cleveland, Ohio

THERE is such a voluminous literature dealing with nephrosis, or chronic parenchymatous nephritis, and there are so many case reports, that one hesitates to add still another to the list. This case, however, is probably worthy of consideration because of the length of time over which observations were continued and the apparent relationship of the urinary P$_H$ and salt and water metabolism.

As is well known, the views of equally competent observers are diametrically opposed when the salt metabolism is investigated. Widal, Abrami, Vallery Radot[1] and their school believe that the kidney is the determining factor in salt retention; that there is a blood salt retention whenever the kidney is unable to excrete the amount of salt ingested, and that by dialysis the excess salt is distributed to the tissues, thus increasing their osmotic power, and causing edema.

Martin H. Fischer[2] believes that the kidney *per se* is not primarily at fault. He teaches that, because of certain changes in the cells and fluid tissues of the body, all of which are colloidal in nature, there is an increased affinity for water and neutral salts, so that these are taken up from any available source. The kidney is involved in this process only as one organ in a widely spread pathologic change. The increaesd salt content of the tissues is a concomitant change; a result of the factors responsible for the tissue changes and not the cause of the edema. There are three types of materials which experimentally can produce these changes. They are acids, alkalies, and amines. Acids are constantly formed in the body as a result of metabolism, and there are finely adjusted mechanisms for their neutralization so that the normal body alkalinity may be preserved. There are, however, many conditions in which there is a profound disturbance of this regulatory mechanism which may be confined to some localized area or may be widespread. In cardiac failure, for example, there is stasis first in the feet and legs as these are farthest from the heart. This stasis prevents a normal supply of oxygen to the tissues with the result that the metabolites are incompletely oxidized and lactic acid is produced in large quantities. This combines with the cell protoplasm, increasing their acid content or perhaps in severe cases their P$_H$. These cells then swell, absorbing water from the vessels near by. Alkali in excess may produce similar effects but this condition is not met with except in cases poisoned by alkalies. The amines, which act similarly, are the most

frequent offenders. Most bacterial toxins are amines, and many are formed when any protein material is broken down.

While these views of Fischer were condemned by many observers when they were first announced, the pendulum of thought is gradually swinging in the direction of their general confirmation. Most clinicians now regard the parenchymatous nephritides as generalized toxemias and Marriott[3] recently stated that Claussen had isolated a toxic substance from the urine and blood of these cases, which would produce salt retention and edema when injected into animals. He stressed the importance of removing all foci of infection and reported cases in which complete cures were obtained by these methods. Eppinger[4] also states that the chief difficulty in the edemata lies in the subcutaneous tissues, and not in the kidneys.

Recently Aldrich and McClure[5] reported their observations on the intracutaneous salt solution disappearance time. Briefly, they found that in individuals with edema, salt solution injected into the skin disappeared in from a few seconds to a few minutes, while in normal individuals it required sixty minutes. They were able to predict clinical improvement with loss of edema whenever the disappearance time increased. These observations point to increased water-holding power of tissue as the cause of edema.

The patient M. L. R., aged twenty-six, was admitted to the medical service of Mount Sinai Hospital from the Out-Patient Department, on February 3, 1924, for a study of salt metabolism in relation to edema. He had previously been hospitalized here from May 15, 1923, until July 7, 1923. On his first admission he gave the following history:

Early in January he noticed a marked swelling of the left submaxillary regions for which he consulted a nose and throat specialist, but for which no local cause could be found. About one week later his feet and legs began to swell. He consulted a physician who told him he had kidney trouble and advised him to take things easy and to restrict his fluid intake. Rest and fluid restriction at home enabled him to return to work, but in ten days the edema recurred and he entered the hospital.

The family history was irrelevant. The previous history stated that he had had some of the diseases of childhood, which ones he did not know, but that there was apparently complete recovery from them. No acute infection preceded the present illness, nor did the patient remember any serious illness. Physical examination revealed a well-developed white male of twenty-five years with slight edema of the face and shins. The eyes, nose, mouth, throat, thorax and abdomen showed no abnormalities. The reflexes were normal. The blood pressure was 140/80. Laboratory examination revealed:

Urine: total volume 1000 c.c., acid, and specific gravity 1.025. Albumin in large amount, sugar negative. Microscopically, a few white blood cells and an occasional cast were found.

Phenolsulphonephthalein test showed 65 per cent in two hours, 70 per cent in three hours. The Mosenthal test showed no fixation of specific gravity. It varied between 1.005 and 1.015 with a night specific gravity of 1.010 and a volume of 480 c.c.

Blood count showed hemoglobin to be 70 per cent, white blood cells 7,800.

Nonprotein nitrogen	32 milligrams per 100 c.c.
Uric acid nitrogen	17
Urea nitrogen	2.4
Creatinine	1.5
Glucose	100
Plasma chlorides	626

Dietary management, reduction of salt and water, together with the use of Novasurol produced marked improvement and he was discharged to the Out-Patient Department on July 7, 1923.

In the dispensary he was instructed to limit his fluid intake to 1,000 c.c. daily and to eat a salt-poor diet. This he faithfully carried out, but in spite of good care, the edema recurred and he was re-admitted to the hospital.
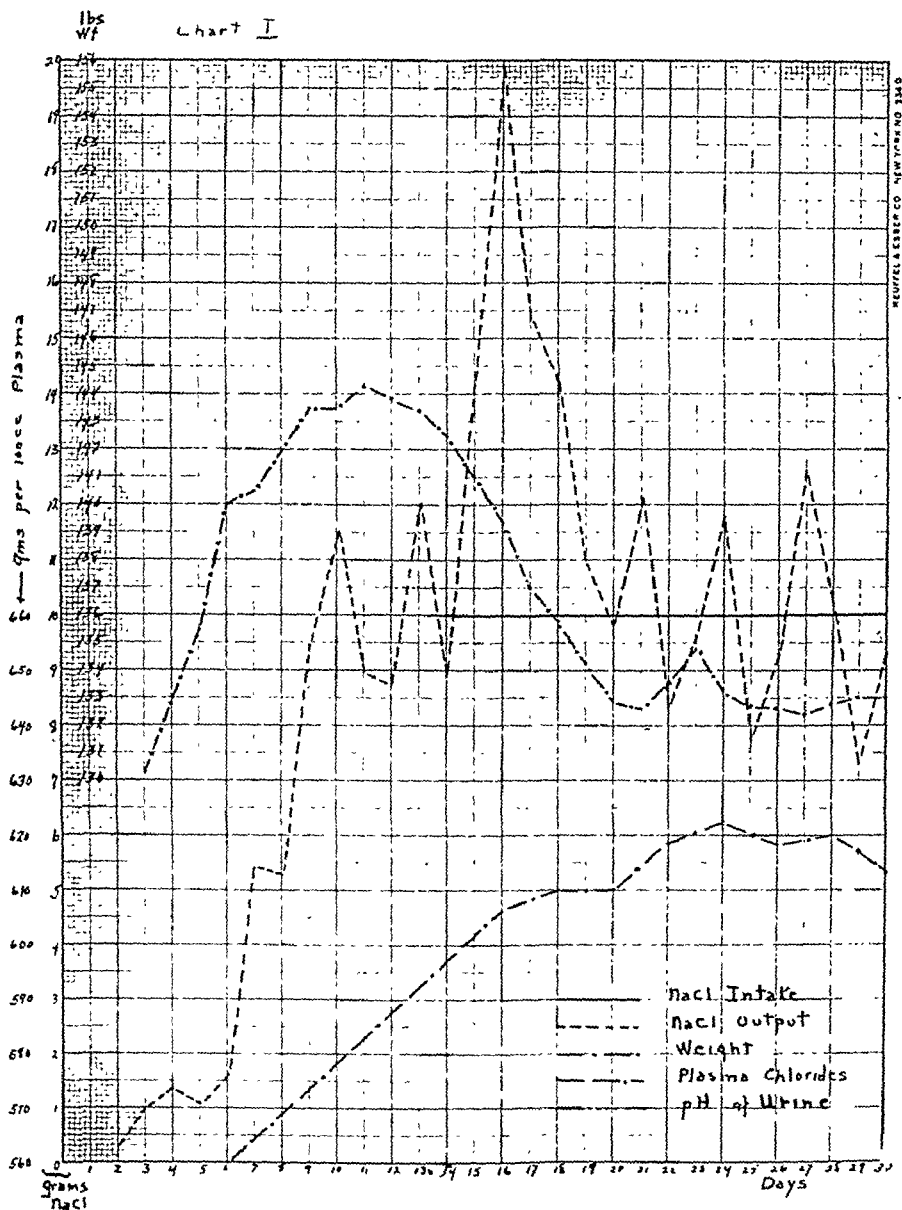
Chart I.

At the time of the second admission the urinary output was 500 c.c. and contained only 0.2 grams of sodium chloride. The plasma chlorides measured 530 mg. per 100 c.c. showing that he had been on a very good salt-free diet. The other findings were comparable to the previous admission. He was placed on a full house diet for twelve days and was then given a full diet containing ten grams of sodium chloride daily.

Records were made of the exact sodium chloride intake, the urinary volume, the percentage of salt output, the total salt output, the weight, the plasma chlorides, and urinary P_H. The charts show in a graphic way the re-

sults obtained. Chart I shows the gradual gain in weight produced when a full house diet, the salt content of which was not known, was substituted for the salt-free diet which he had been taking for several months. This rise was paralleled by a rise in total sodium chloride output which reached 12 grams. A diet containing exactly 10 grams of salt was then begun. There was a sharp rise in total sodium chloride excretion with a sharp loss of weight. The
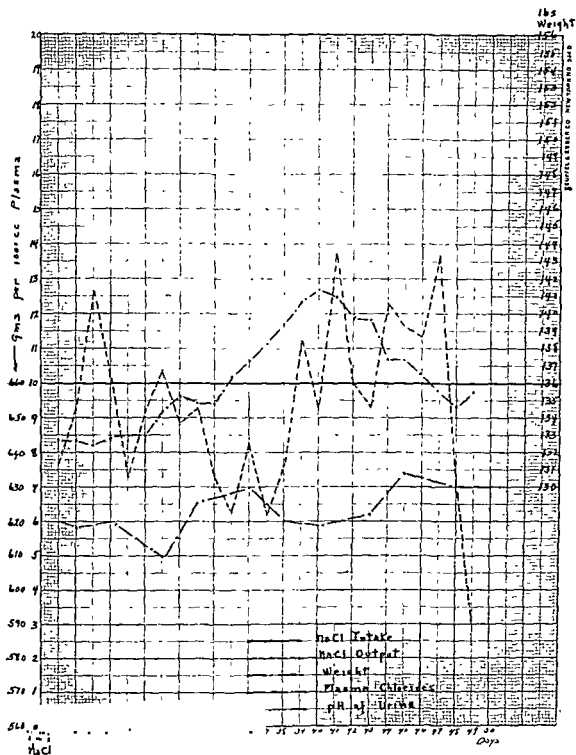
Chart II

salt excretion and intake then practically balanced each other but the weight fluctuated as much as two pounds. The plasma chlorides rose gradually from 560 to 620 mg. per 100 c.c.

Chart II shows remarkable variatons in weight and in sodium chloride excretion despite the stable intake of 10 grams of sodium chloride. The weight fluctuated a maximum of nine pounds and sodium chloride output a maximum of 7 grams. Obviously there must be some factors other than salt

intake which determine the salt output and the weight. In examining the urine it was noted that during the sharp rises in weight with decreased salt output, the urine was highly acid; accordingly the P_H of every specimen voided was determined colorimetrically and the average for the day plotted in relation to the other curves. Charts III and IV show the marked urinary acidity coincidental with the diminished salt output and the gains in weight. The urine P_H was between 6.6 and 7.0, the chloride output between 11.5 and 13.6 grams, and the weight 134½ pounds. There was then an abrupt decrease in urinary P_H with a marked decrease in the percentage of sodium chloride output and a sharp drop in the total number of grams of sodium chloride. When
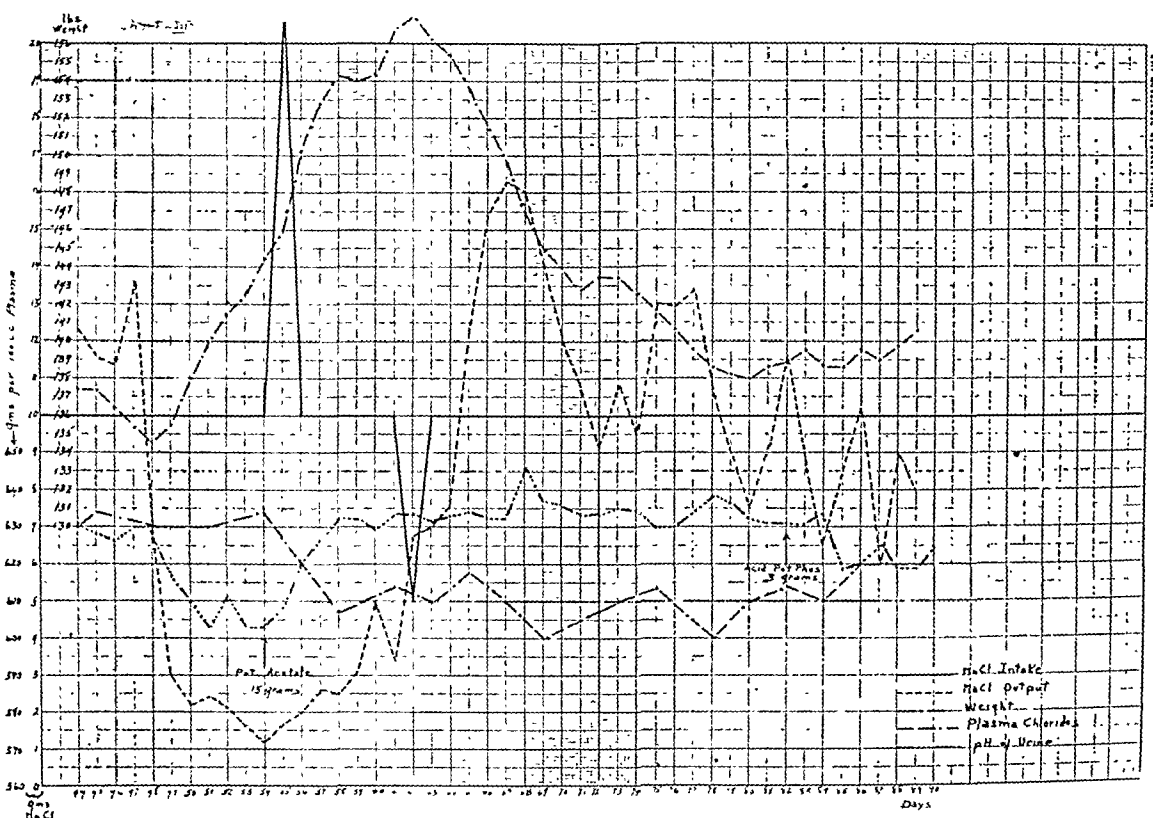
Chart III.

the urinary P_H reached 4.4, the volume was 330 c.c., the sodium chloride 0.4 per cent and the total sodium chloride fell to 1.2 grams.

At this point 15 grams of potassium acetate were administered daily in an attempt to reduce the urinary acidity. It required seven days to bring the urine to a neutral reaction. As the acidity lessened the first noted change was a marked increase in the per cent of sodium chloride excreted; the second, an increase in urinary volume and of course a corresponding increase in total sodium chloride excreted. The weight continued to rise, reaching a maximum of 158 pounds before the total sodium chloride excretion reached 10 grams and balanced the intake. In other words it required six days after the chloride output was stimulated for the step-like rise described by Vallery Radot to take place, to effect a balance between intake and output. While the urinary P_H was maintained at 7.0 or above there was a progressive loss of fluid until

138 pounds weight was reached. At this point the urinary P$_H$ was reduced to 6.0 and a slight gain in weight was recorded. The weight of 138 pounds represented a clinical condition similar to that on admission. There was no edema of the face, but slight pitting over the shins. There had been a physiologic gain of weight. During these observations, estimations of the carbon dioxide combining power of the plasma were carried out every other day. There were no significant variations.
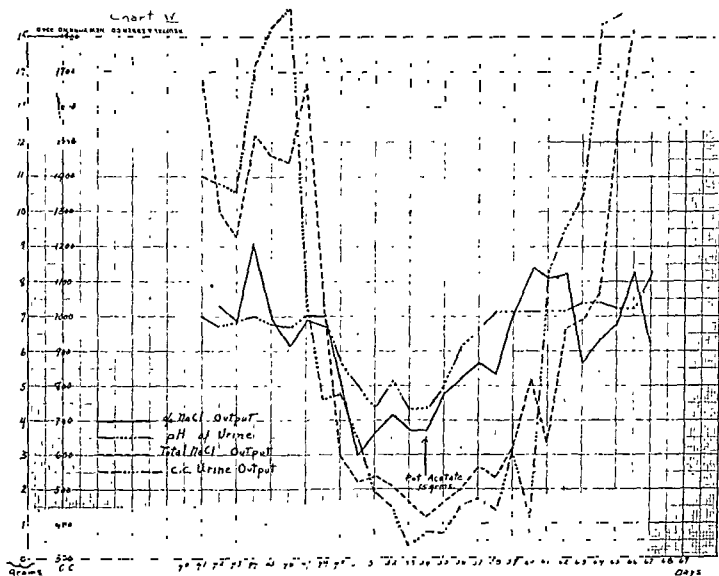
Chart IV.

SUMMARY

Records of the urinary volume, per cent chloride output, total sodium chloride output, urinary P$_H$, plasma chlorides and weight were kept for 110 days in a case of nephrosis on a diet containing 10 grams of sodium chloride daily.

With a rise in weight there was a chloride retention, diminished water output, and a heightened acidity of the urine. Administration of alkalies to render the urine neutral was accompanied by a sharp increase in chloride output and loss of edema. Administration of acid potassium phosphate reversed the process and was followed by a gain in weight.

I wish to express my thanks to Dr. S. S. Berger for his kindness in permitting me to make the observations on this case.

REFERENCES

Marriott, McKim: Nephritis in Children. Med. Clin. N. Amer., March, 1924, vii, 1413-1419. St. Louis Number.
Aldrich, C. A., and McClure W. B.: Time Required for Disappearance of Intradermally Injected Salt Solution. Jour. Am. Med. Assn., July 28, 1923, lxxxi, 293-294.
Vallery-Radot, Pasteur: Rythme en Échelons de la retention chlorurée. Presse méd. Paris, 1918, xxvi, 135-137.
Fischer, Martin H.: Oedema and Nephritis, 1918, Ed. 3, John Wiley and Son, N. Y.
Eppinger: Die Nephritis Frage, 1924, Ed. 2, Perles Co., Vienna.

ENDO'S MEDIUM*

By C. C. YOUNG, D.P.H., AND M. S. MARSHALL, M.A., LANSING, MICHIGAN

AFTER a special culture medium has been developed for a particular use, modifications begin. The medium is tried for purposes for which it was not intended. variations are made which simplify or improve it for the original use. and changes creep in the technic of preparation or in the formulae which are often insidious. The principle is certainly commendable, but the result is that the new worker is lost in the variations, and the more experienced worker is unsettled as to which variations are preferable, allowable, and advantageous. It is to be hoped that the manual of culture media in preparation under the auspices of the Society of American Bacteriologists, under the guidance of Dr. Buchanan, and with the help of the Digestive Ferments Company, will have a tendency to stabilize such tendencies and overcome such duplication of work.

It is the purpose of the present paper to summarize briefly some of the chief Endo medium variations, and to present experimental data which point out evidence on the whole confirming some points brought out by other authors, although not agreeing with all reported work.

In Table I are charted the formulae for the more common "Endo" media.

There is agreement on the amount of peptone and the amount of lactose throughout; in all other ingredients there is variation. Perhaps the most used variation from the original Endo medium is Kendall's modification, which was based on the necessity for a rapid diagnosis of bacillary dysentery; Endo's original medium was made for typhoid diagnosis.

The significant points may be given as:

1. Variation in the percentage of agar: increase in percentage decreases the *rate* of diffusion of the color-changing substances. Hence a low percentage is advantageous for rapid work, but dangerous if used where the time of reading is not under careful control. Variation is useless without careful P_H control, for a neutral medium will change color more rapidly than an alkaline medium.

2. Use of salt: not of material importance, unless there be some slight effect upon agglutinative properties of the organisms, which is doubtful.

TABLE I

SUMMARY OF "ENDO" MEDIA

#	ENDO[1]	KEN-DALL[2]	K. AND D.[3]	R. AND R.[5]	L.[8]	P. AND W.[4a]	STITT[21]	APHA[22]
Agar	3.0	1.5	1.5	2.5	1.5-3.0	2.5	3.0	3.0
Peptone	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.5	–	0.5	–	–	–	0.5	–
Lactose	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Beef ext.	–	0.3	0.3 or	0.5	–	0.5	0.5	0.5
Beef inf.	+	–	±	–	–	–	–	–
Fuchsin x	0.5	0.09	0.25	0.5	0.5	0.5	0.6 b	0.5
Na_2SO_3 y	2.5	0.9	–	–	2.5	–	2.0 b	2.5
$NaHSO_3$ z	–	–	0.5	1.0	–	1.0	–	–
Px	7.8 c	7.0 d	7.6-7.8	8.0 e	no adj.	8.2	8.0 f	"as required"
K_2HPO_4	–	–	–	–	0.2-0.5	–	–	–

Figures represent percentages.
x Saturated alcoholic solution.
y 10 per cent aqueous, fresh.
z 10 per cent aqueous, fresh.
a As Robinson and Rettger,[5] except for PH.
b 6 drops per 100 c.c ; 20 drops per 100 c.c.

c Neutral to litmus + 1 per cent of a 10 per cent Na_2CO_3.
d Just alkaline to litmus.
e +0.1 per cent : 6.8 + 1 per cent of a 10 per cent Na_2CO_3.
f 0 0 or + 0.2 per cent.

3. Use of beef extract: most authors have adopted the more convenient meat extract instead of using fresh meat infusion. This point is questionable, since we are dealing in a diagnostic laboratory with somewhat delicate pathogenic forms not of optimum viability.

4. Variations in amounts of fuchsin and sodium sulphite. Much obviously depends on these quantities.

5. The P_H of the media: this has been stated to be important,[3, etc.] and should with the advent of P_H control methods be consistently established.

Harding and Ostenberg,[19] DeBord,[11] and Kahn,[14] have given experimental evidence of the nature of the Endo reaction. The color of the fuchsin-sulphite indicator is restored by organic acids and aldehydes, and by acetone and alcohol. These may be produced in different amounts and in different ratios by different organisms. It has been the general experience by those who have used Endo's medium in routine examinations of feces that the identical medium will present somewhat different aspects when inoculated with different fecal flora. The ideal Endo medium will differentiate the typhoid, paratyphoid, or dysentery cultures in the presence either of weak or of violent color-producers.

Experiments were made with a view to checking the use of beef extract and beef infusion, the amounts and ratio of basic fuchsin and sodium sulphite, and the P_H. Preliminary experiments were made with media containing beef extract or beef infusion, with saturated alcoholic basic fuchsin in varying amounts up to 0 5 per cent, each combined with varying amounts of sodium sulphite up to 3.5 per cent of a 10 per cent fresh aqueous solution. These combinations were tried at P_H values from 6.5 to 7.7. Four feces were inoculated to plates of each combination of variables, using *B. typhosus* and *B. dysenteriae* cultures in dilute suspension in the feces.

The results showed that Endo's original formula gave the largest typical colonies, the least diffusion, and the best differentiation. This medium has a

beef infusion agar base, uses 0.5 per cent fuchsin solution with 2.5 per cent sodium sulphite solution, and has a P_H of from 7.5 to 7.7.

To secure unbiased results from these media from routine work, six different combinations of fuchsin and sulphite were outlined, from 0.05 per cent to 1.00 per cent fuchsin solution, and from 0.50 to 5.00 per cent sodium sulphite solution, all at a P_H of 7.5 to 7.7, and with either a beef extract or a beef infusion base. These were supplied under a code number by the media division of the laboratory to the diagnostic division for a period exceeding one month,* the latter reporting their results daily. Freshly isolated *B. typhosus* and *B. dysenteriae* were used in control plates throughout.

The results were in favor of beef infusion agar, with 0.2 to 0.5 per cent fuchsin solution and 1.0 to 2.5 per cent sodium sulphite solution, the two in a respective ratio of 1:5.

Different concentrations of a 1:5 fuchsin sulphite medium, representing the above concentrations, were added to an Endo base without lactose. Pure cultures of *B. coli*, *B. typhosus*, and *B. dysenteriae*, all freshly isolated from feces, were inoculated to the plates.

TABLE II

EFFECT OF DECOLORIZED FUCHSIN ON SIZE OF COLONY

Basic fuchsin sol.	0.1	0.3	0.5	1.0	1.5 per cent
Sodium sulphite sol.	0.5	1.5	2.5	5.0	7.5 per cent
B. coli, 1st trial	1.7*	2.0	2.0	1.7	2.0 mm.
2nd trial	2.5	2.5	2.0	2.0	2.0
B. typhosus, 1st trial	1.0	0.8	0.5	0.8	0.5
2nd trial	2.0	1.5	1.0	0.7	0.5
B. dysenteriae, 1st trial	1.0	1.5	1.0	0.7	0.5
2nd trial	2.5	2.0	1.5	0.7	0.0

*Diameter of colonies at 18 hours in mm.

The bacteriostatic action of the fuchsin is evident on *B. typhosus* and *B. dysenteriae*, but we have seen no evidence that the original Endo amounts of fuchsin-sulphite mixture are seriously inhibitive, although a slightly smaller amount has given satisfactory differentiation and growth.

It has been objected that fuchsin and sulphite vary in different lots of chemicals; the procedure of Kastle and Elvove, titrating fuchsin with sulphite to a maximum sensitivity with formaldehyde, would overcome such difficulty. There has been, however, no cause for worry on this point in our experience.

SUMMARY

A brief summary of the most common Endo media in use is given, with references.

A brief write-up of an extended series of feces examinations by Endo media of varying meat base, P_H, and fuchsin-sulphite amounts and ratio is given.

The conclusions of Gilbert and Coleman based on 4,552 examinations, namely, that the original Endo medium is as satisfactory as any for typhoid,

*NOTE: 1280 feces examinations were made at this laboratory during the fiscal year 1923-1924.

paratyphoid, and dysentery diagnosis, and more satisfactory than some, is endorsed. In justice to the various writers discussed, further conclusions will be left to the reader, as each writer has directed his attention to his own problems.

Acknowledgment is made to Miss Lucy Dell Henry, who handled all of the media and a considerable part of the experimental work.

REFERENCES

[1]Endo: Centralbl. f. Bakteriol., 1904, xxxv, Part I, p. 109, Endo's original paper.

[2]Kendall and Day: Jour. Med. Research, 1911, xxv, 95. Kendall's modification.

[3]Kligler and Defendorfer: Jour. Bacteriol., 1918, iii, 437. Modification.

[4]Park and Williams: Pathogenic Microorganisms.

[5]Robinson and Rettger: Jour. Med. Research, 1916, xxix, 363. Modification.

[6]Ayers and Rupp: Jour. Infect. Dis., 1918, xxiii, 188. Reversal of Reaction in Colon-Typhoid Group.

[7]Kligler: Jour. Exper. Med., 1918, xxviii, 319.

[8]Levine: Am. Jour. Pub. Health, 1918, viii, 864. Modification.

[9]Wilson: Jour. Hygiene, 1923, xxi, 392. Chemistry of reaction.

[10]Kligler: Jour. Exper. Med., 1917, xxvii, 463. Sensitivity of B. Dysenteriae.

[11]DeBord: Jour. Bacteriol., 1917, ii, 309. Chemistry of reaction.

[12]Ayers and Rupp: Jour. Bacteriol., 1918, iii, 433. Colon-aerogenes medium.

[13]Teague: Jour. Am. Med. Assn., 1918, lxx, 454. Sterilized Na_2SO_3 under oil

[14]Kahn: Jour. Bacteriol., 1918, iii, 547. Chemistry of reaction.

[15]Kahn: Am. Jour. Pub. Health, 1919, ix, 373. Endo medium slants.

[16]Bronfenbrenner and Schlesinger: Proc. Soc. Am. Bacteriol., Abst. Bacteriol., 1919, iii, No. 14. Substitution of indicator.

[17]Gilbert and Coleman: Proc. Soc. Am. Bacteriol., Abst. Bacteriol., 1922, vi, No. 95. Experience with 4552 feces examinations.

[18]Burke and Skinner: Jour. Exper. Med., 1924, xxxix, 613. Bacteriostatic action of fuchsin.

[19]Harding and Ostenberg: Jour. Inf. Dis., 1909, xi, No. 1. Chemistry of reaction.

[20]Kastle and Elvove: Jour. Infect. Dis., 1909, xvi. Technic of preparation

[21]Stitt: Practical Bacteriology.

[22]Standard Methods of Water Analysis, Am. Pub. Health Assn.

TREATMENT OF NASAL DIPHTHERIA CARRIERS*

By Clara Israeli, M.D., Manchester, N. H.

THE carrier problem in infectious diseases is a difficult one, because clinical symptoms are lacking or are of such mild character that a correct diagnosis is not made. It is only after cultural study that a diagnosis is made. This is especially true of diphtheria and typhoid carriers.

In the case of throat diphtheria carriers, tonsillectomy has been resorted to, but this practice is not applicable to nasal diphtheria carriers. Treatment with mild antiseptic douches and sprays in nasal diphtheria cases has not given satisfactory results. There are rhinologists who are so radical as to state that no treatment is of avail.

In diphtheria we are dealing with a local infection of the mucous membrane. Other organisms, having already made the mucous membranes vulnerable or actually diseased, favor the growth of diphtheria bacilli. The latter cause excoriation of the mucous membrane and bleeding. With such a pathologic condition, it seemed to us that if the mucous membrane could be healed with some substance which would at the same time destroy the organisms, the results would be favorable. Since antiseptic solutions have not filled the need, it seemed to us that ointment might be worthy of trial. For this purpose a ten per cent ichthyol ointment was used. It is a mild antiseptic, allays inflammation, aids in destroying the microorganisms and thus favors healing of the mucous membrane.

The first case treated was a child two years old, who had a nasal discharge and an alternate daily positive culture. To establish the site of infection, cultures were taken from the nose, throat and ears. There was a discharge from the ears. The nasal culture alone showed diphtheria organisms.

The method used was as follows: A piece of absorbent cotton was wound tightly on the end of an applicator, and as much of the ointment as would hold on the end of the swab was carried gently all the way into the nasal cavity, entering the nasopharynx; another swab is used in the same way in the other nasal orifice.

This method of treatment was carried out five consecutive days. Nasal discharges ceased. Treatment was omitted for two days. A culture was taken on the third day. Growth was scant, with a few staphylococci, but no diphtheria bacilli. Cultures were taken on three consecutive days, all giving negative results. The patient was discharged. A culture taken a month later gave negative results.

The next were two cases—sister and brother—admitted to the hospital,

	HOME	PRELIMINARY CULTURE	NO. OF DAYS TREATED	NO. OF DAYS OMITTED	CULTURE AFTER TREATMENT	RESULTS	SECOND WEEK TREATMENT	DAYS OMITTED	CULTURE	RESULTS	SECOND DAY CULTURE	THIRD DAY CULTURE	THIRD DAY	CULTURE ONE MONTH LATER	
1. R. D. 4 months	Some Time	Nose + and Throat -	5 days	2 days	Third day	Diphtheria almost pure culture	5 days	2 days	3rd.	Scant growth. Few diplococci. No diphtheria	Scant growth. No diphtheria	Growth scant. No diphtheria	Patient discharged	Negative for diphtheria	
2. J. Dr. Coburn's case	Some Time	Nose + and Throat -	5 days	2 days	Third day	Diphtheria	5 days	2 days	3rd.	Growth diphtheria	Treatment 5 days	Omitted 2 days	Culture Third day	Negative for Diphtheria	Second and third day cultures were negative for diphtheria

NO.	NAME OF PATIENT	HOSPITAL	PRELIMINARY CULTURE	NO. OF DAYS TREATED	NO. OF DAYS OMITTED	DAY OF CULTURE TAKEN	RESULTS	SECOND DAY CULTURE	THIRD DAY CULTURE	CULTURE ONE MONTH LATER	TOTAL NO. OF DAYS IN HOSPITAL
1.	W. K., 2 yrs. 2 wks.		Nose + Throat − Ears −	5 consecutive	2 days	Third day	Scant growth few diplococci. No Diph.	Scant growth. No Diph.	Scant growth no diph. Patient discharged	Negative results	24 days
2.	A. Z.	On day admitted	Nose + Throat − Ears 0	5 days	2 days	Third day	Few diplococci. No Diph.	Few diplococci. No Diph.	No Diphtheria. Patient discharged		10 days
3.	M. Z.	On day admitted	Nose + Throat − Ears 0	5 days	2 days	Third day	Few diplococci. No Diph.	Few diplococci. No Diph.	No Diphtheria. Patient discharged		10 days
4.	L. G.	On day admitted	Throat − Nose + Ears 0	5 days	2 days	Third day	Few diplococci. No Diph.	Few diplococci. No Diph.	No Diphtheria. Patient discharged		9 days

having been isolated on culturing the school for diphtheria carriers. These two cases had a severe nasal discharge with bleeding, excoriated mucous membrane. Cultures taken from the nose and throat showed the nose alone to be the site of infection. There was no discharge from the ears, so cultures were omitted. Treatment was carried out five consecutive days. Nasal discharge ceased, the mucous membrane healing well. Treatment was omitted for two days. A culture was taken on the third day. Growth showed few staphylococci, no diphtheria bacilli. Cultures were taken on three successive days, all giving the same amount of growth, but no diphtheria organisms. The patients were discharged.

The next case was a child four months old which was cared for at home. The culture showed a persistent nasal diphtheria infection almost in pure culture. The people objected to quarantine and to having the physician take so many cultures. The health department sent a nurse to carry out the treatment according to the above method. At the end of the first week when the first culture was submitted to the laboratory, the growth was in almost pure culture, proving that treatment was not carried out faithfully. Another week was used in treating this case under specific directions, with ten per cent ichthyol ointment. At the end of the week a culture submitted to the laboratory, showed scanty growth, with a few diplococci, but no diphtheria bacilli. Cultures were taken on three successive days. The growth was the same. The patient was discharged and quarantine removed. A culture taken a month later gave negative results.

Several other cases were treated according to this method with satisfactory results. A few physicians who had private cases with persistent nasal diphtheria, have on our recommendation, used this method with satisfactory results. Some of the physicians submitted cultures to us for control while they had their patients treated. It was found that it required three weeks to a month to obtain as completely successful results as obtained by us.

The cases under our observation did not show any signs of sinusitis.

COMMENT

We present this short report because we have found the treatment successful. It is simple, inexpensive, and easily applied. As a rule the time is comparatively short, depending, of course, on the faithfulness of the applications. We reiterate this point, because, as we have indicated above, by our control work, it was shown, that the longest time required for successful results was in cases where treatment was haphazard. It is fair to say that treatment was not carried out by the physicians, but left to a nurse.

The cases treated by us, and those treated by other physicians have not so far as we have been able to determine, been a source of infection, either in their homes or in school. They would have again come under our notice as they did in the first place.

THE RELATION OF THE GALL BLADDER TO THE DEVELOPMENT OF JAUNDICE FOLLOWING OBSTRUCTION OF THE COMMON BILE DUCT*

By Frank C. Mann, M.D., and Jesse L. Bollman, M.D., Rochester, Minn.

IN our studies on the effect of total removal of the liver, we noted that jaundice developed in the hepatectomized animal. The urine secreted shortly after operation was bile-colored. Within six hours, the plasma became yellow, and the jaundice increased progressively until death. If animals lived sixteen hours or more a definite icteric tinge developed in the sclerotic and mucous membranes. At necropsy all the fatty tissues were a dirty yellow. This yellow pigment which accumulated in the urine, plasma and fat reacted positively to the accepted tests for bilirubin, thus definitely proving the liver is not essential for the formation of bile pigment.

A comparison of our data on the formation of bilirubin after hepatectomy, with certain observations on jaundice following occlusion of the common bile duct, showed that there was seemingly a discrepancy between the rate of development of jaundice after hepatectomy and after common bile duct obstruction. When the common bile duct of a dog was obstructed, a demonstrable amount of pigment did not appear in the blood for many hours, whereas when the liver was removed, the pigment appeared within a few hours. This seemed to imply that there was an increase in formation of bilirubin after hepatectomy, and might imply an increase in blood destruction. However, the red cells and hemoglobin did not show an increase in blood destruction after hepatectomy, but remained practically normal. In order to determine the reason for this discrepancy between the rate of accumulation of bilirubin in the blood following hepatectomy, and that following occlusion of the common bile duct, as well as to obtain data with regard to the rate of formation of bilirubin with the liver intact as compared with the rate after hepatectomy, a series of experiments was performed.

PREVIOUS WORK ON THE CONCENTRATING ACTIVITY OF THE GALL BLADDER

One of us has recently published a review of the literature on the functions of the gall bladder, so that literature related to the subject will only be discussed briefly. Kemp called attention to the difference between the character of the bile secreted by the liver and that found in the gall bladder, and to the inspissated character of the bile in the gall bladder. Bidder and Schmidt found, experimentally, that much fluid is absorbed from the bile in the gall bladder. Hohlweg demonstrated that by the gall bladder's absorption of water; it could concentrate the bile secreted during the intervals between digestion, so that the 40 c.c. of bile found in the gall bladder represented from 240 to 400 c.c. of bile from the liver. Rous and McMaster ligated the

common bile duct of dogs, and the bile secreted by a portion of the liver went into the gall bladder, while that secreted by the remainder of the liver passed into the bile duct, to which a rubber balloon was attached. Control observations were made on the uniformity of the pigment content of the bile secreted from different portions of the liver. It was found that the pigment content of each cubic centimeter of bile in the gall bladder might be ten times greater than the bile secreted into the duct, showing that the bile in the gall bladder had been greatly concentrated. Afanassiew observed that jaundice developed much more rapidly in animals when the biliary tract was filled with wax than when the common duct alone was occluded. Bloom, in studying the rôle of the lymphatics in the absorption of bile pigment from the liver in cases of early obstructive jaundice, found that bilirubin appeared in the blood about two hours after ligation of the common bile duct and exclusion of the gall bladder, while positive tests for the pigment were obtained on the lymph from the same animal from forty-five to sixty-two minutes after ligation.

METHOD

All the experiments were performed on dogs; all operative procedures were performed under ether anesthesia, employing aseptic technic. In one group of animals the common bile duct was sectioned between clamps and doubly ligated; the remaining portion of the biliary tract was left intact. In the other group of animals the common bile duct was obstructed in the same manner, and the gall bladder was removed. Observations were made on the time of appearance and rate of accumulation of bile pigment in the plasma and urine of the two groups of animals. The van den Bergh method was employed for the quantitative estimation, as well as qualitative tests.

RESULTS

The results of the experiments are very striking (Fig. 1). Animals in which the common bile duct is obstructed and the gall bladder left intact do not have an appreciable amount of bile pigment in the urine for from thirty-six to forty-eight hours after operation; only a faint trace of bilirubin, if any, is found in the plasma at the end of twenty-four hours. The scleras do not develop an icteric tinge for from forty-eight to seventy-two hours. While the various animals differ slightly, it is usually forty-eight hours between the time of obstruction of the common bile duct, and the appearance of the first definite evidence that jaundice is developing. The subsequent development of icterus and the accumulation of bilirubin in the blood is also slow. Well defined icterus is usually not observed for more than forty-eight hours after the first appearance of bile pigment in the blood. When the gall bladder is removed at the same time the duct is ligated, the length of time between operations and the first appearance of jaundice is greatly decreased. Bile pigment appears in the urine and a definite test for bilirubin is obtained in the plasma within from three to six hours after the ligation of the common duct and the removal of the gall bladder. The amount of pigment in the plasma increases rapidly, and the jaundice is fully developed within twenty-four to forty-eight hours. Before the end of the first twenty-four hours the

sclerotics and mucous membranes are definitely icteric, and there is no question about the animal being jaundiced.

The great difference between the rate of the development of jaundice in animals in which the common bile duct is obstructed and those in which the gall bladder is removed and the same obstruction produced, is illustrated very clearly by comparing the evidence of icterus in the two series. The sclerotics and the mucous membranes in the animals of the first series appear normal for from three to four days after operation, while those of the second series are definitely icteric after twenty-four hours. The urine of the animals of the first series remains free from bile pigment for from thirty-six to forty-eight hours, while the second series contains large amounts within three to six hours, and is found in all urine passed after that time. Comparison of
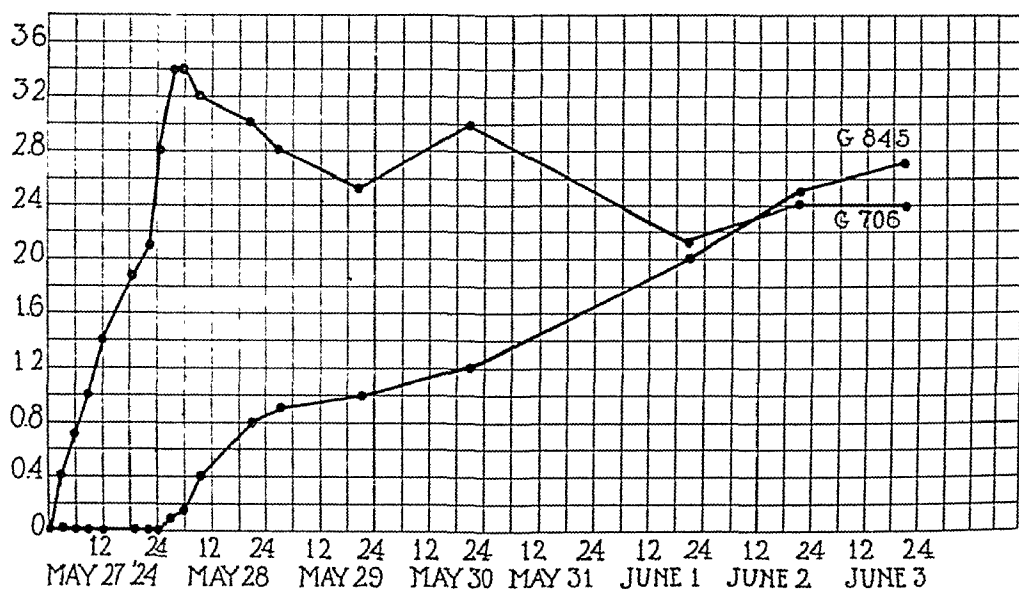
Fig. 1.—Curve showing the accumulation of bilirubin in the plasma of two dogs following obstruction of the common bile duct. At *0* both animals had their common bile duct sectioned and ligated. In Dog G845 the remainder of the biliary tract was not disturbed, while in Dog G706 the gall bladder was removed. The figures are given in van den Bergh units.

the plasma of the two series from three to thirty-six hours after operation gives a clear colorless plasma in the first, and a definitely yellow plasma in the second. From thirty-six to eighty-four hours after operation the plasma of the first series is a pale yellow, while that of the second is a deep yellow. The van den Bergh test on these plasmas demonstrates even more clearly the slow development of bilirubin in the first series, compared with the rapid development in the second series. The direct reaction is given by the van den Bergh test only after thirty-six to forty-eight hours in the first series, and within three to six hours in the second series. In all cases in which the indirect reaction was positive, the direct reaction was also positive, although it was somewhat delayed on the plasma containing small amounts of pigment.

DISCUSSION

The marked difference in the rate of the accumulation of bile pigment in the animals in which the common bile duct is obstructed, and in those with

similar obstruction and removal of the gall bladder illustrates strikingly the remarkable activity of the gall bladder. Inspection of the plasma or serum obtained from these animals any time from three to forty-eight hours after ligation of the common bile duct, shows clearly which animals are without gall bladders, and which have normally functioning gall bladders. This experiment could be utilized as a classroom demonstration to show that the gall bladder has a function. The same difference in time of appearance of jaundice is noted if the cystic duct is ligated, and the gall bladder allowed to remain intact. Moreover, if the gall bladder is severely injured, the same rapid accumulation of pigment follows ligation of the common duct. The same rapid appearance of pigment occurs after ligation of the common duct in animals that have been subjected to cholecystectomy several weeks previously, resulting in greatly dilated hepatic and common ducts. This fact eliminates, to a considerable degree, the mechanical factor which might otherwise be considered, that the elasticity of the gall bladder prevents the early accumulation of pigment when the gall bladder is present in obstructed animals.

SUMMARY

Following ligation of the common bile duct, signs of icterus, that is, bile pigment in the urine, plasma, sclerotics and mucous membranes, do not develop for from thirty-six to forty-eight hours after operation. If the gall bladder is removed when the common duct is ligated, the urine and plasma contain definite amounts of bile pigment within three to six hours after operation, and the sclerotics and mucous membranes are definitely yellow within twenty-four hours. The difference in the rate of accumulation of bile pigment is considered as evidence of the ability of the gall bladder to concentrate the bile secreted by the liver.

REFERENCES

[1] Afanassiew, M.: Ueber Icterus und Hämoglobinurie, hervorgerufen durch Toluylendiamm und andere Blutkörperdien zerstörende Agendien. Ztschr. f. klin. Med., 1883, vi, 281-331.

[2] Bidder and Schmidt: Quoted by Beale, L. S.: On some points in the anatomy of the liver of man and vertebrate animals. London, J. Churchill, 1856, p. 68.

[3] Bloom, W. J.: The rôle of the lymphatics in the absorption of bile pigment from the liver in early obstructive jaundice. Bull. Johns Hopkins Hosp., 1923, xxxiv, 316-320.

[4] Hohlweg, H.: Ueber Störungen der Salzsäureabscheidung des Magens bei Erkrankungen und nach Exstirpation der Gallenblase. Deutsch. Arch. f. klin. Med., 1912, cviii, 255-276.

[5] Kemp, G.: Experimental researches on the function of the mucous membrane of the gall bladder, principally with reference to the conversion of hepatic into cystic bile. Proc. Roy. Soc. Med., 1856-1857, viii, 133-140.

[6] Mann, F. C.: The functions of the gall bladder. Physiol. Reviews, 1924, iv, 251-273.

[7] Mann, F. C., Bollman, J. L., and Magath, T. B.: Studies on the physiology of the liver IX. The function of bile pigment after total removal of the liver. Am. Jour. Physiol., 1924, lxix, 393-409.

[8] Mann, F. C. and Magath, T. B.: The effect of total removal of the liver. Tr. Sect. Path. and Physiol. Am. Med. Assn., 1921, 29-42.

[9] Rous, P. and McMaster, P. D.: The concentrating activity of the gall bladder. Jour. Exper. Med., 1921, xxxiv, 47-73.

[10] van den Bergh, A. A. H.: La recherche de la bilidubin dans le plasma sanguin. Presse méd., 1921, i, 441-443.

THE EFFECT OF SPECIFIC CHOLECYSTITIS ON THE BILE-CONCENTRATING ACTIVITY OF THE GALL BLADDER*

By Jesse L. Bollman, M.D., Frank C. Mann, M.D., and Pierre DePage, M.D.,
Rochester, Minn.

IN another paper we have shown that the presence of the gall bladder is sufficient to prevent bile pigment from appearing in the blood or urine of a dog[3] for more than twenty-four hours following ligation of the common bile duct. If the gall bladder is removed, or the cystic duct ligated, at the time the common bile duct is ligated, bilirubin appears in the blood and urine within three hours after obstruction of the common bile duct. With this method for testing the bile-concentrating activity of the gall bladder, we studied the effect of a specific cholecystitis on this activity. One of us has called attention to the chemical means of producing a specific cholecystitis by the intravenous injection of sublethal amounts of chlorinated soda (Dakin's solution). In this manner an acutely inflamed gall bladder may be produced almost immediately following injection, with but slight coincident changes in other organs. The lesions in the gall bladder may persist for a number of weeks after all evidence of disturbance has disappeared from other organs. This observation has been confirmed, in experiments on the dog, by Peterman, and by Sweet and Reimann. Stenhouse reported several cases of cholecystitis in man which seem to be the result of inhalation of poison gas.

METHOD OF RESEARCH

Dogs were used in the experiments. Three or four days previous to operation, the animals were injected intravenously with 10 c.c. for each kilogram of body weight, of a solution of chlorinated soda (Dakin's solution). Double ligation of the common bile duct with section of the duct between the ligatures was performed under ether anesthesia with aseptic technic. The remaining portion of the biliary tract was not disturbed. The gall bladder was examined *in situ*, and the extent of the gross lesions recorded. Observations were subsequently made on the time of appearance and rate of accumulation of bile pigment in the plasma and urine. The animals were examined at necropsy to make certain that the ligated bile ducts had not ruptured. The van den Bergh method was employed for the detection of bilirubin in the plasma.

RESULTS

There was a surprising parallelism between the time of appearance of bile pigment in the plasma and the gross appearance of the gall bladder at the time of operation. The various animals developed bile pigment in the plasma and urine at widely different times, and the pigment accumulated in the blood

at widely different rates in accordance with the variations in extent of the lesions in the gall bladder. The tests of some animals for bilirubin in the blood and urine were positive as early as three hours after ligation of the common bile duct, and the accumulation of bile pigment in the blood was rapid, so that the animals appeared jaundiced in less than twenty-four hours. A similar picture is obtained following ligation of the common bile duct and extirpation of the gall bladder. All of the animals that developed bile pigment in the blood and urine so rapidly following ligation had been observed to have a most severe inflammation of the gall bladder at the time of operation, the entire organ appearing to be involved.

In another group of animals the tests for bilirubin in the blood or urine were not positive until twenty-four to thirty-six hours after ligation of the
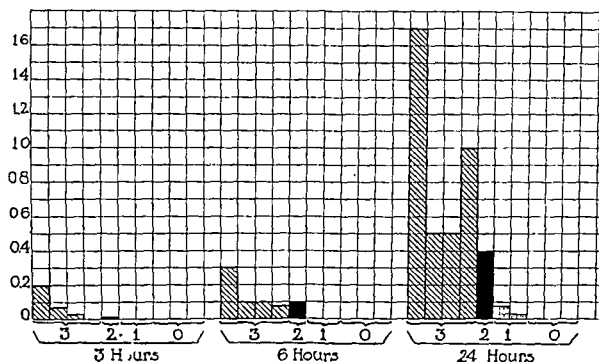
Fig. 1.—Chart showing the development of jaundice in relation to the severity of lesions of the gall bladder. Estimations of plasma bilirubin three, six and twenty-four hours after ligation of the common bile duct in animals with a normal appearing gall bladder, 0; in animals with a very mild degree of cholecystitis, 1, in animals with a moderate degree of cholecystitis, 2; and in animals with a very severe cholecystitis, as judged by inspection at the time of operation, 3.

common bile duct. This finding is similar to that following ligation of the common bile duct in an otherwise normal animal. In these animals, no lesion of the gall bladder was observed at the time of ligation of the common bile duct.

The remaining animals of this series developed bilirubin in the blood and urine at periods between the extremes mentioned. Animals with more severely inflamed gall bladders showed signs of jaundice earlier than those with milder and less extensive lesions (Figs. 1 and 2).

DISCUSSION

The activity of the gall bladder in concentrating the bile is clearly shown to be impaired by inflammatory lesions of the gall bladder. If these lesions are extensive, involving almost the entire organ, this activity is apparently

entirely lost.   The functional significance of this activity of the gall bladder is by no means certain, but it is easily disturbed by the presence of acute lesions in the organ.   It will be interesting to determine the permanent effect of an acute inflammatory process on this activity, and the influence of a chronic process, with a view to ascertaining whether or not the activity returns with the subsidence of the inflammatory process.
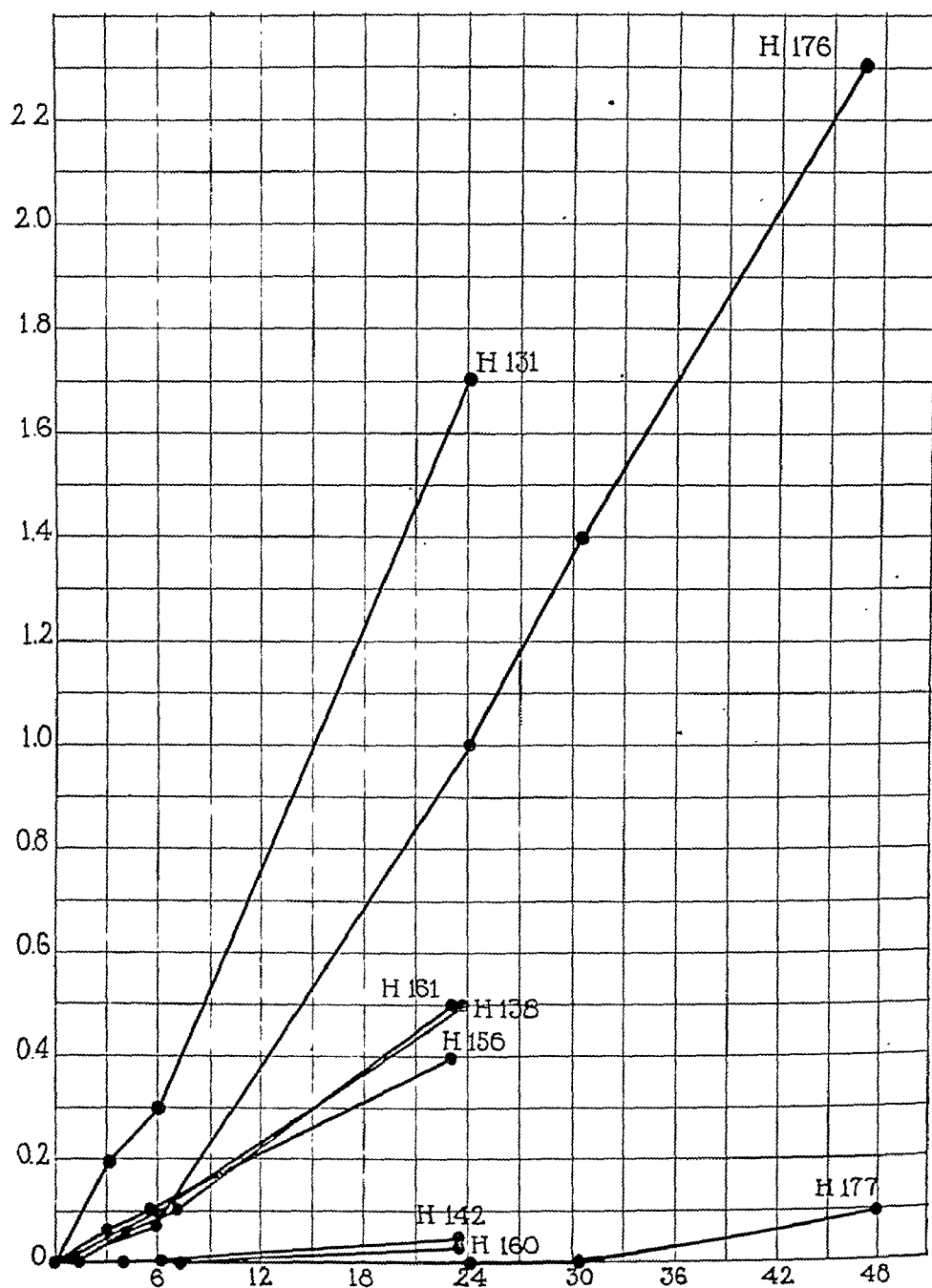
Fig. 2.—Curve showing the development of bilirubin in the plasma following ligation of the common bile duct.   Dogs H131 and H176 had a severe cholecystitis involving the entire gall bladder; in Dogs H161, H138, and H156 about one-half of the gall bladder appeared to be involved; in Dogs H142 and H160 the gall bladder was but slightly involved;   Dog H177 was a normal animal.   The bilirubin estimations are in van den Bergh units.

SUMMARY

A specific cholecystitis has been produced in dogs by the intravenous injection of solutions of chlorinated soda. The time of appearance and rate of development of bile pigment in the blood and urine of these animals following ligation of the common bile duct depends on the degree of involvement of the gall bladder by this acute process. In animals in which the whole gall bladder seemed diseased, jaundice developed as rapidly as in animals in which the gall bladder was removed, and the common bile duct ligated similarly. Animals having milder disease of the gall bladder developed jaundice less rapidly, and animals with but slight or no apparent disease developed jaundice at the same rate as normal animals following ligation of the common bile duct. These facts clearly demonstrate that the function of the gall bladder of concentrating the bile is impaired, or may be totally lost, in the presence of an acute inflammatory process in the gall bladder.

REFERENCES

[1] Deaver, J. B. and Reimann, S. P.: Cholecystostomy versus cholecystectomy. Jour. Am. Med. Assn., 1924, lxxxii, 2115-2117.
[2] Mann, F. C.: The production by chemical means of a specific cholecystitis. Ann. Surg., 1921, lxxiii, 54-57.
[3] Mann, F. C., and Bollman, J. L.: Relation of the Gall Bladder to the Development of Jaundice Following Obstruction of the Common Bile Duct, JOUR. LAB. AND CLIN. MED., April, 1924, x, No. 7, p. 540.
[4] Peterman, M. G.: Cholecystitis and its complications. Surg, Gynec and Obst., 1923, xxxvi, 522-541.
[5] Stenhouse, H. M.: Cholecystitis of chemical origin in man following inhalations of poison gas. U. S. Nav. Med. Bull., 1923, xix, 291-296.
[6] van den Bergh, A. A. H.: La recherche de la bilirubin dans le plasma sanguin. Presse méd., 1921, i, 441-443.

ON THE APPLICATION OF THE HENCH-ALDRICH UREA INDEX TO THE SPINAL CORD[*]

By George S. Graham and Sarah H. MacCarty

A RAPID method for the determination of the urea content of the saliva has recently been developed under Rountree's direction by Hench and Aldrich.[1] It is based on Friedlander's titrimetric method for the estimation of the urea content of the urine. It requires for its application only the simplest of apparatus and reagents, and yet appears capable of returning to the clinician information of considerable value. The method depends upon the fact that urea combines with mercury salts to form stable compounds. The amount of urea contained by a given solution will therefore be proportional to the amount of mercury bound by it. The test consists in adding from burette a 5 per cent solution of mercury bichloride to 5 c.c. of saliva and determining by an indicator solution of sodium carbonate the point at which the mercury-combining power of the salivary constituents is satisfied and free mercury ions remain in the solution. The amount of mercury solution calculated as necessary to saturate 100 c.c. of saliva is taken as the "Urea Index." Certain other substances are capable of binding mercury salts. Most important of these biologically are ammonium salts, uric acid, creatinine and amino acids. Hench and Aldrich found in practice that the mercury bound by all salivary bodies other than urea and ammonia nitrogen was, in normal individuals, 39 per cent of the total amount taken up; in patients with urea retention, 35 per cent. The test was found to have a dependability of about 90 per cent. That is, with normal values for the salivary urea index there was, in nearly 90 per cent of cases, no urea retention in the blood. With a salivary index above the normal, definite urea retention was proved in 91 per cent of cases. In urea retention there was a close parallelism between the figures for the salivary index and those of the blood urea as determined by the urease method.

During the past winter the method was taken up in this laboratory. We were soon convinced that the claims made for its simplicity and usefulness were borne out in practice. The question then arose as to the applicability of the method to the determination of the urea content of the spinal fluid. The body membranes are highly permeable to urea and the substance is rather evenly distributed throughout the tissues and fluids. On the other hand, the choroid plexus holds back from the spinal fluid a considerable percentage of other substances capable of combining with mercury. Theoretically the spinal fluid should show a more highly specific urea-combining value than does the saliva. Myers and Fine[2] found that while the urea

content of the spinal fluid was 88 per cent that of the blood, the creatinine was only 46 per cent, and the' uric acid but 5 per cent. There is, however, some difference of opinion as to the actual relationship existing between the urea content of the spinal fluid and that of the blood. Woods[3] gives the two values as approximately the same. Myers and Fine[2] found considerable variation in different cases. In four instances from a series of fourteen cases, the spinal fluid urea was from 100 per cent to 121 per cent that of the blood; in six cases it was from 90 per cent to 95 per cent; in three cases, from 81 per cent to 87 per cent; and in one case, 69 per cent. Cullen and Ellis[4] made a series of determinations on thirty-two patients, all of whom appear to have been suffering from tabes. In 63 per cent of their cases the difference between the urea content of blood and of spinal fluid was less than 2 milligrams per 100 c.c. The greatest difference found was 11 milligrams. The urea values varied from 20 to 42 and from 22 to 46 milligrams per 100 c.c. for serum and spinal fluid respectively.

From these and similar studies it would appear that in most cases the spinal fluid contains urea in a concentration approximating that of the blood. A method adapted to measuring this urea simply and rapidly should have considerable clinical value. Routine determinations in diagnostic lumbar puncture fluids might give incidental information as to urea retention and might occasionally point the way to diagnosis in otherwise obscure cases of uremic coma. With such considerations in mind, the attempt has been made to find out whether the Hench-Aldrich test may be expected to give results for the spinal fluid as dependable as those claimed for it in the saliva. In a small series of cases the mercury has been determined in parallel with urease determinations on spinal fluid and blood. In a larger series it was not practicable to run urease determinations. Here the mercury index alone was obtained. These simple figures are not, however, without some interest. In a few cases the test was applied to spinal fluids obtained at autopsy. The technic used throughout was the same as that of the original method, the only change consisting in the substitution of 5 c.c. of spinal fluid for 5 c.c. of saliva. The controlled urea index findings are given in the following table:

TABLE I

CASE	SEX	AGE	DIAGNOSIS	MERCURY UREA INDEX SP. FLUID	UREA N BY UREASE, SP. FLUID	UREA N BY UREASE, BLOOD
1	F	30	Myocarditis	28	8.47	10.7
2	M	35	Syphilis	36	–	13.9
3	M	28	Syphilis	45	18.75	20.4
4	M	62	Lymphatic leucemia	48	15.7	18.48
5	M	50	Brain tumor (?); Lues (?).	50	16.7	18.48
6	M	39	Fracture of skull	64	19.05	–
7	M	52	Cardiorenal disease	128	46.8	54.35
8	M	30	Sickle cell anemia; streptococcic pneumonia	153	58.35	–
9	M	60	Myocarditis; bronchopneumonia	260	119.0	128.0
10	M	54	Prostatic hypertrophy	258	73.65	78.6

The results in these few cases seem sufficiently clear to warrant further trial of the method. For the first eight cases there is approximate agree-

ment between the values obtained by the mercury titration and by the urease method. As between the last two cases, a difference of 45 milligrams in the urease levels is associated with a difference of only 2 in the urea indices. It is probable that the discrepancy here is due to a distortion of the index in Case 10. During titration a heavy white curdy precipitate appeared. Smaller amounts of such a precipitate have been seen not uncommonly. It has been particularly noticeable in postmortem spinal fluids. When present in abundance the substance or substances producing it may act as a buffer or as a direct binder of the mercury and so defer the reaction between mercury and urea. It is possible that a perfected technic may serve to eliminate this possible source of error.

Concerning the fundamental question as to the relative concentration of urea in the spinal fluid and in the blood, our average results agree with those of Myers and Fine. We have not encountered so wide a range of variation as appeared in their series. Cullen and Ellis, Woods and earlier writers appear to have obtained percentages more constant than our own. There is evident need here for further study, particularly with a view to determining whether the ratio may vary in different types of disease. The question has, of course, a practical bearing for the present study, since upon its answer must depend much of the clinical value of any method such as that now proposed.

In a series of twenty unselected cases, the urea index was taken as a single factor unsupported by more exact chemical determinations. The lowest value, 34, was obtained in a case of tetanus in a nine-year-old child. Three patients gave a value of 36 and six gave values of 36 to 50. In two of the latter group there was some urinary evidence of renal disturbance. In one, a forty-five-year-old man, under intraspinal treatment for cerebrospinal syphilis, three tests covering a period of one month gave indices of 40, 50 and 45. The urine showed a trace of albumin but no casts. In the other, a man of twenty-three years suffering from acute Staphylococcus meningitis, an index of 48 was accompanied by a trace of albumin in the urine. In general it may be said that urinary or other evidence of nephritis begins to appear in the series at a urea index level of about 50. A case of chronic suppurative mastoiditis in a man of twenty-five years with an index of 50 showed urinary albumin and a few hyaline casts. On the other hand, urinary findings were negative in a case of tabes dorsalis with an index of 52.5 and again in a case of cerebrospinal syphilis in which five tests taken over a period of seven weeks gave indices of 54, 54, 68, 50 and 50. · An index of 55 in an eleven-months-old infant that died twenty-four hours later of streptococcic meningitis could not be checked. In a nine-months-old infant with "breast milk poisoning," spinal fluid obtained one hour before death gave an index of 62.5 and the urine showed a trace of albumin, many hyaline casts and a 4-plus acetone reaction. An index of 63 in a seventy-six-year-old man with prostatic hypertrophy was accompanied by a urinary specific gravity of 1005, a faint trace of albumin, and occasional hyaline casts. Three cases gave indices of 100 to 116.6. One of these was a child of four years with acute suppurative otitis media and bronchopneumonia; the urea index was 116.6.

The urine showed a specific gravity of 1021; a trace of albumin; pus content rated as 2-plus; there were many hyaline, granular and cellular casts. The other two cases of high index figures were adult males with obvious renal disease.

Spinal fluid was obtained at autopsy in seven cases. The mercury index here was usually high and had obviously no relationship with antemortem urea retention. The lowest value, 46, was found in the fluid from a child of four years who died of hydrocephalus consequent upon a large solitary tubercle of the cerebellum. The fluid was obtained twelve hours after death. A value of 85 was obtained twenty-four hours after death in a man fifty years of age who died three hours after fracture of the skull. The kidneys showed only microscopic sclerosis. Four cases had indices of 112 to 204 without anatomic lesions sufficient to explain a urea retention. The highest value, 737.5, was obtained in a two-months-old infant that died of acute purulent meningitis. Centrifuged fluid was used but there was long-persisting formation of a curdy precipitate as the mercury was added.

In three cases we had the opportunity of examining the fluid both before and after death. In one, Case 9 of Table I, the index was 260, ten hours *before death* and 428, *eight hours after death*. In a second case of hydrocephalus, an index of 40 was obtained two days before death. Seven hours after death, fluid from the lateral ventricle gave an index of 78. In a case of tuberculous meningitis an index of 64 on the second day preceding death had changed to one of 208 eighteen hours after death. Such findings are in agreement with those of Myers,[5] *who showed that no value could attach to postmortem study of the spinal fluid.* It is probable that waste and disintegration products begin to accumulate during the agonal period and that they mount rapidly immediately after death.

SUMMARY AND CONCLUSIONS

An attempt has been made to apply the Hench-Aldrich test for salivary urea to the determination of the urea content of the spinal fluid. The procedure is simple and a determination can be made in a few minutes. It is not an exact chemical method comparable to the well-established procedures commonly employed and it is still questionable how far it may be expected to agree with these. The work is being continued with the view to answering this question. The indications are, however, that it will prove sufficiently delicate and reliable to detect any distinct urea retention in the body fluids and that it may be expected to give an approximate determination as to the degree of this retention. Its possible value as a useful sign in obscure cases of uremic coma is evident. The normal urea index appears to range from about 30 to about 50.

REFERENCES

[1] Hench, P. S., and Aldrich, M.: Jour. Am. Med. Assn., 1923, lxxxi, 1997; ibid, 1924, lxxxii, 1194.

[2] Myers, V. C., and Fine, M. S.: Jour. Biol. Chem., 1919, xxxvii, 239.

[3] Woods, A. C.: Arch. Int. Med., 1915, xvi, 577.

[4] Cullen, G. E., and Ellis, A. W. M.: Jour. Biol. Chem., 1915, xx, 511.

[5] Myers, V. C.: Jour. Biol. Chem., 1909, vi, 115.

THE READING OF COMPLEMENT-FIXATION TESTS BY THE CITRON SCALE AS COMPARED WITH A METHOD USING A COLOR SCALE[*]

By Ruth Gilbert, M.D., A.M., Margaret F. Kelley, A.B., M.A., and Anna C. Moore, A.B.

THE scale of reading complement-fixation tests quite generally adhered to in this country is an adaptation of the Citron scale,[1] in which the fixation obtained in the two amounts of serum tested is recorded as follows:

1. Fixation complete with both 0.02 c.c. and 0.01 c.c. of serum = 4+
2. Fixation complete with 0.02 c.c. and nearly complete with 0.01 c.c. = 3+
3. Fixation complete with 0.02 c.c. and partial with 0.01 c.c. = 2+
4. Fixation nearly complete with 0.02 c.c., partial or slight with 0.01 c.c. = ÷
5. Fixation partial or slight with 0.02 c.c., partial or slight with 0.01 c.c. = ±
6. Complete hemolysis in both tubes = no complement fixation obtained.
   There are two objections to this scheme:

1. This scale of values has been found misleading because of the wide range of ÷ from nearly complete fixation to only partial fixation, so that a reaction which may be relatively strong is reported as only + and is frequently interpreted as having no clinical significance.
2. The scheme is not uniformly applied by different workers because of the variations in the individual's conception of the terms, nearly complete, partial or slight fixation.

It, therefore, seemed desirable to adopt a scale in which a more definite valuation would be given to sera giving nearly complete fixation in the larger amount, and to establish this scale upon standards which could be more uniformly applied. With this end in view, the Citron method of reading was compared with a method in which the exact degree of inhibition of hemolysis was estimated by comparison with color standards containing varying percentages of hemolyzed and nonhemolyzed cells.

The use of such a color scale for reading complement-fixation tests was advocated as early as 1910 by Thomsen and Boas,[2] working in Madsen's laboratory. Verne,[3] in France, devised an ingenious artificial scale for this purpose. Bergeron and Normand[4] found, however, that the colors in the Verne scale did not exactly correspond to the color of hemolyzed cells, so they introduced a natural scale using varying percentages of hemolyzed cells. In this country, Mahr[5] has reported on the use of an artificial color scale, and Terry[6] on a natural one made up with varying proportions of cells and hemoglobin solution. Ivy[7] has recommended the use of the Duboscq colorimeter for estimating the degree of hemolysis, which is an accurate but complicated method. Kolmer[8] also recommends the use of a color scale and points out that it should contain, in addition to the varying proportions of sheep's corpuscles and

patient's serum was tinged with hemoglobin, a control was made containing the amount of patient's serum used in the test, 0.2 c.c. of sensitized cells and 0.85 per cent salt solution, to bring the volume up to 0.5 c.c. This was centrifugalized and the color of the supernatant liquid compared with that of the color standards. The per cent hemolysis to which this control corresponded was subtracted from that shown by the test itself, the difference being the actual per cent hemolysis shown by the serum in question.

Comparison of Results with Two Methods of Reading.—In order to compare the results of these two methods of reading, it was necessary to translate the amounts of inhibition of hemolysis observed by the use of color standards into the degrees of definite and partial fixation represented by the terms $\pm$, $+$, $2+$, $3+$ and $4+$. The scale tentatively adopted, based on the 0.02 c.c. amount of serum, was that sera showing from 10 to 20 per cent inhibition of hemolysis should be considered $\pm$; those showing from 25 to 45 per cent inhibition $+$; those from 50 to 70 per cent inhibition $2+$; those from 75 to 95 per cent inhibition $3+$; and those showing 98 to 100 per cent inhibition $4+$. This seemed the most logical scale, and the clinical data of the borderline cases indicated that it was safe to place the lower limit of definite fixation at 50 per cent inhibition of hemolysis.*

TABLE I

COMPARATIVE RESULTS OF 2051 COMPLEMENT-FIXATION TESTS WITH THE PLAIN ALCOHOLIC ANTIGEN READ BY THE CITRON METHOD AND BY A METHOD USING A COLOR SCALE

CITRON METHOD	PERCENTAGE INHIBITION OF HEMOLYSIS IN 0.02 C.C. SERUM					
	TOTAL	10-20 ($\pm$)	25-45 (+)	50-70 (2+)	75-95 (3+)	98-100 (4+)
Total	2051	177	178	119	424	1153
$\pm$	376	177	159	40	0	0
+	245	0	19	78	134	14
2+	21	0	0	1	17	3
3+	145	0	0	0	115	30
4+	1264	0	0	0	158	1106

TABLE II

CLINICAL HISTORIES OF THE 266 CASES IN WHICH COMPLEMENT-FIXATION TESTS MADE WITH PLAIN ALCOHOLIC ANTIGEN WERE READ AS + OR $\pm$ BY THE CITRON METHOD AND 4+, 3+ OR 2+ BY THE USE OF A COLOR SCALE

NUMBER OF SPECIMENS	CLINICAL HISTORY
266	Total Cases
178	Treated cases of syphilis
27	Untreated cases diagnosed as syphilis
26	Untreated cases with history or symptoms suggestive of syphilis
2	Cases with immediate members of family syphilitic
5	Cases without definite history but stated to have had a previous blood test giving definite complement fixation
21	Cases without definite history but giving definite complement fixation with the cholesterinized antigen by both methods of reading
7	Cases with data inadequate for classification
1	Acquired syphilis? Weakness, 5 years
1	Arthritis
1	Headache 4 years
1	Auditory neuritis
3	No history given

*It was found in a small series of comparative readings that, in grouping the results according to this scale, it was sufficient to use only the limiting standards of 25 per cent, 50 per cent, 75 per cent and 95 per cent inhibition of hemolysis.

TABLE III

COMPARATIVE RESULTS OF 2331 COMPLEMENT-FIXATION TESTS WITH CHOLESTERINIZED ANTIGEN READ BY THE CITRON METHOD AND BY A METHOD USING A COLOR SCALE

CITRON METHOD	PERCENTAGE INHIBITION OF HEMOLYSIS IN 0.02 C.C. SERUM					
	TOTAL	10-20 (±)	25-45 (+)	50-70 (2+)	75-95 (3+)	98-100 (4+)
Total	2331	72	154	109	351	1645
±	242	72	138	32	0	0
+	320	0	16	77	199	28
2+	37	0	0	0	20	17
3+	131	0	0	0	67	64
4+	1601	0	0	0	65	1536

Two thousand and fifty-one comparative readings were made with the plain alcoholic antigen. The comparative results of these tests by the two methods of readings are shown in Table I. Inasmuch as approximately 12.5 per cent of the tests read 4+ by the Citron method actually showed from 5 to 20 per cent hemolysis, it is evident that this degree of hemolysis is not always recognized with the cells in suspension. There is also an error in the other direction, as approximately 6 per cent of the tests read + by the Citron method showed no hemolysis in the 0.02 c.c. amount of serum Though there would have been some redistribution of the tests which read 4 , 3 - or 2+ by the Citron method on the basis of the color standard readings, they would all have been reported as giving definite fixation of complement by either method of reading. However, of the 621 tests read + or ± by the Citron method, 266, or slightly over 40 per cent, were read 2+, 3+ or 4+ with the use of a color standard. The clinical histories of these 266 cases are given in Table II.

Two thousand three hundred and thirty-one comparative readings were made with the cholesterinized antigen. The comparative results of these tests by the two methods of reading are given in Table III These results likewise indicate the errors in reading tests with cells in suspension, since in approximately 4 per cent of the cases read 4+ by the Citron method, there was actually from 5 to 20 per cent hemolysis, and almost 9 per cent of those which read + showed no hemolysis in the larger amount when read with a color standard. Of the 562 tests which read + or ± by the Citron method, 336 or approximately 60 per cent were read 2+, 3+, or 4+ with the use of a color standard. The clinical histories of these 336 cases are given in Table IV.

An analysis of the histories given in Tables II and IV indicates that reading complement-fixation tests by the use of color standards, with the range of 50 to 100 per cent inhibition for definite fixation, gives results more in conformity with the clinical findings than those obtained by the use of the Citron scale.

Summary and Conclusions.—The readings of tests after centrifugalization by recording the percentage inhibition of hemolysis as compared with a color standard brings out the fact that the Citron method of reading with the cells in suspension does not, in many instances, *give an exact estimate of the amount of fixation that has occurred.* Comparative readings have shown that from 5 to 20 per cent hemolysis is frequently not recognized with the cells in suspension, so that sera showing less than 20 per cent hemolysis in both

TABLE IV

CLINICAL HISTORIES OF THE 336 CASES IN WHICH COMPLEMENT-FIXATION TESTS MADE WITH THE CHOLESTERINIZED ANTIGEN WERE READ AS + OR ± BY THE CITRON METHOD AND 4+, 3+ OR 2+ BY THE USE OF A COLOR SCALE

NUMBER OF SPECIMENS		CLINICAL HISTORY
336		Total Cases
238		Treated cases of syphilis
31		Untreated cases diagnosed as syphilis
21		Untreated cases with history or symptoms suggestive of syphilis
2		Cases with immediate members of family syphilitic
5		Cases without definite history but stated to have had a previous blood test giving definite complement fixation
39		Cases with data inadequate for classification
	1	Trifacial neuralgia
	1	Acquired syphilis? Weakness, 5 years
	1	Dizziness, two weeks
	1	Weakness and dizziness
	1	Pneumonia, two weeks
	1	Rash on limbs
	1	Endarteritis of leg
	1	Indolent ulcer foot and hands, 1 month
	1	Delayed healing of wound
	1	Paralysis agitans
	1	Headache, six weeks
	1	Headache and nervousness, not considered syphilitic by the attending physician
	1	Psychosis, case from insane hospital
	1	Prostitute, involutional melancholia, no physical signs of syphilis
	1	Manic depressive insanity and history of three miscarriages; attending physician considered it unlikely that lues entered into etiology
	1	Negress, syphilis denied
	1	Inmate of state prison, venereal disease denied
	3	Inmates of the state prison, syphilis denied, gonorrhea admitted
	19	No history given

amounts are read 4+, but with 20 per cent or less hemolysis in the 0.02 c.c. amount and 30 per cent or more in the 0.01 c.c. amount are usually read +, making the apparent difference in the reading greater than the actual difference in degree of fixation. The Citron method of reading is misleading because the reports on two specimens which give nearly complete fixation in the larger quantity of serum, may vary between 4+ and +, whereas careful comparison with a color standard, using the scale of reading proposed, would result in reports on all such specimens of definite complement fixation with a quantitative difference based on the percentage inhibition of hemolysis observed. This would tend to make more uniform the results reported on successive specimens from the same patient and the results reported by workers in different laboratories. The scale adopted results in an increase in definite reactions and a decrease in the partial reactions which are so misleading and confusing to the clinician. At the same time the limits have been so placed as to avoid giving too great significance to weak or slight fixation.

REFERENCES

1Citron, J.: Immunity, Methods of Diagnosis and Therapy. Trans. by Garbat, A. L., Ed. 2, 1914.

2Thomsen, O.: Die quantitative Ausführung der Wassermannschen Reaktion. Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, vii, 389.

3Vernes, A.: Indices syphilimétriques. Détermination colorimétrique. Compt. rend. Acad. d. sc., 1918, clxvii, 500.

[4]Bergeron, A. and Normand, E.: De l'emploi d'une échelle colorimétique naturelle dans la réaction de Bordet-Wassermann. Presse méd., 1918, xxvi, 472.

[5]Mahr, E. F.: Studies in the Serology of Syphilis, Syphilimetric Color Indices. Jour. Lab. and Clin. Med., 1921, vii, 1.

[6]Terry, B. T.: A Method for Standardizing the Reading of the Wassermann Reaction, with Demonstration. Proc. N. York Path. Soc., 1917, xvii, 117.

[7]Ivy, R. M.: The Duboseq Colorimeter as a Means of Estimating the Hemolysis in the Wassermann Reaction. Jour. Am. Med. Assn., 1912, lix, 432.

[8]Kolmer, J. A.: Studies in the Standardization of the Wassermann Reaction. A Study of Methods for Conducting Quantitative Complement-Fixation Tests and of Reading Scales for Recording Reactions. Amer. Jour. Syph., 1922, vi, 64.

THE CHLORIDE CONTENT OF THE BLOOD IN PERNICIOUS ANEMIA*

By Russell L. Haden, M.D., Kansas City, Kansas

CERTAIN facts suggest a possible relation of chloride metabolism to pernicious anemia. Free hydrochloric acid is practically constantly absent from the gastric juice in this disease. The presence of free acid makes the diagnosis of pernicious anemia extremely questionable. Levine and Ladd[1] found an achlorhydria in 104 of 105 patients. In Cabot's series[2] 78 of 79 patients showed an absence of free hydrochloric acid. I have reported[3] 50 cases in 47 of which a gastric analysis was done and free acid found absent in every instance. It is highly probable that the occasionally reported finding of free hydrochloric acid represents an error in technic or in diagnosis.

Pernicious anemia is a hemolytic disease of toxic origin. The gastrointestinal tract is probably the site of origin of the toxin. Some observers hold that the absence of free hydrochloric acid is the immediate cause for the development of the toxin. Others with equally good reasons think that the disappearance of free acid is a result and not a cause. According to the latter view, the achlorhydria, the blood destruction, the bone marrow changes, the glossitis and the characteristic nervous system lesions are all manifestations of the action of an unknown toxin.

The toxin responsible for the toxemia of high intestinal obstruction is possibly closely related to the toxin of pernicious anemia. Numerous cases have been reported in which a blood picture indistinguishable from idiopathic pernicious anemia has occurred with either a benign or a malignant stenosis of the small intestine.[4] Such seemingly represent cause and effect rather than coincident diseases. The fundamental and characteristic feature of the chemical pathology of high intestinal obstruction is a disturbance of chloride metabolism.[5] The well-recognized occurrence of a blood picture resembling pernicious anemia in certain cases of carcinoma of the stomach is also of interest in this connection. It is highly probable that in such cases there is a toxin elaborated identical with or similar to that responsible for idiopathic pernicious anemia. Emerson[6] found that in carcinoma of the stomach there are substances in the gastric contents which tend to

neutralize hydrochloric acid. Since these substances are destroyed by heat they are most probably of the nature of ferments.

A final point in the similarity of the toxemia of pernicious anemia and intestinal obstruction is the occurrence of melanuria in both conditions. Melanuria, in the absence of a melanotic tumor, as Haden and Orr[7] have shown, is indicative of specific type of protein destruction occurring only after the depletion of the blood chlorides between a certain level.

To determine if possible whether a disturbance in chloride metabolism can be found in pernicious anemia, I have made chloride determinations on the blood of all patients with pernicious anemia seen during the past two years. For comparison with these findings, determinations have also been made on numerous cases of secondary anemia.

Other workers have made observations on the chlorides in anemia. Host[3] determined the blood chlorides in several cases and found the plasma content about that of normal blood while the whole blood content tended to be higher. Buckman and Edwards[9] found no depression of chloride content of the red blood cells peculiar to pernicious anemia. They obtained low values for chlorides in all types of anemia with very low cell volumes. Essen and Porges[10] found that the sodium chloride of the blood in anemia lay at the upper level of normal and tended to fall as the anemia improved. Christian[11] has investigated the renal response to the nephritic test meal in pernicious anemia. He found a very low excretion of nitrogen and salt and concludes that this is due to an impairment of kidney function. In cases in which successive tests were made with an improvement in the anemia, there was a marked rise in salt excretion usually out of proportion to the increase in nitrogen. It seems quite possible that the changes in salt excretion observed may represent a disturbance in chloride metabolism rather than in kidney function.

In the cases reported here the chloride determinations were made as part of a complete blood study. Since chlorides are usually much higher in plasma than in corpuscles it is necessary to know the relative cell and plasma volumes. The cell volume in each case was determined by centrifuging 10 c.c. of blood with 2 c.c. of 1.6 per cent (isotonic) solution of sodium oxalate. The volume index was calculated in each instance and reported elsewhere.[5] The chloride determinations were made on the tungstic acid filtrate by iodometric titration after the method suggested by Gettler.[12]

The results in thirty consecutive cases of pernicious anemia are tabulated in Table I. The highest chloride value is 620 mg. per 100 c.c. and the lowest 320 mg. The cases in which there is a low cell volume have higher whole blood chlorides than those in which the cell volume appears normal. The plasma chlorides are all within normal limits. The constancy of the plasma chlorides explains the higher values in bloods with low cell volumes since the chloride content of plasma is usually about 100 mg. per 100 c.c. higher than that of whole blood.

The total blood volume in anemia is little different from normal so there is actually a larger amount of circulating chloride per kilogram of body

weight in individuals with low cell volumes than in normal persons. There is no demonstrable relation of the volume index to the chloride content. In four cases of pernicious anemia (Nos. 22, 23, 28, 30) the whole blood chlorides are below the accepted lower limit of normal.

For comparison with the results in pernicious anemia I have tabulated in Table II in a similar manner the findings in twenty cases of secondary anemia. The results here are in general the same as in pernicious anemia. With low cell volumes the whole blood chlorides are higher than normal while the plasma content is within normal limits. Where the cell volume deviates little from normal, the chlorides show little change.

TABLE I

CHLORIDE CONTENT OF THE BLOOD IN PERNICIOUS ANEMIA

CASE NO.	RED BLOOD CELLS IN MILLIONS PER C.MM.	VOLUME OF RED BLOOD CELLS		VOLUME INDEX	CHLORIDES AS NaCl (MG. PER 100 C.C)		FREE HCL IN GASTRIC JUICE
		PER CENT OF WHOLE BLOOD	PER CENT OF NORMAL		PLASMA	WHOLE BLOOD	
1	0.39	5	10	1.34		550	
2	0.59	8	17	1.42		600	0
	3.21	35	73	1.14		500	0
3	0.60	7	15	1.25	570	530	0
4	0.84	13	27	1.61	640	540	0
5	0.86	12	25	1.46	580	570	0
6	0.87	10	21	1.21		620	0
7	0.98	13.5	28	1.27	560	550	0
8	0.98	13.5	28	1.43		620	0
	0.98	14	29	1.45		520	
9	0.99	13.5	28	1.38		530	0
10	0.39	14	29	1.46		580	?
11	1.04	15.5	32	1.52	580	520	0
12	1.18	15	31	1.32	520	510	0
	0.97	13	27	1.38	570	530	
	2.67	31	65	1.23	575	510	
	4.60	44	95	1.03		445	
13	1.27	20	42	1.27		530	?
14	1.33	17	35	1.32	550	520	0
15	1.35	22	46	1.70	630	495	0
16	1.39	18	37	1.32		520	0
17	1.39	21	44	1.56	560	470	0
18	1.40	20	42	1.50		435	0
19	1.48	22	46	1.53	580	520	0
20	1.52	23	49	1.61		490	0
21	1.87	27	56	1.50	540	480	?
22	1.87	24.5	51	1.36	580	550	
	2.43	29	60	1.22		320	
	3.52					330	
	3.07	39	81	1.01		370	
	3.14	29	60	0.95		405	
23	2.18	27	57	1.31		400	0
	1.63	21	44	1.33	565	500	
	2.42			1.69	580	490	
24	2.23	36	75	1.69	570	480	0
25	2.41	37	76	1.57		420	0
	3.21	32	86	1.35	570	480	0
26	2.60	36	75	1.44	585	500	0
27	2.70	39	80	1.48	625	465	0
28	2.88	41	85	1.46		370	0
29	3.16	44	92	1.46		440	0
30	3.44	36	75	1.09		410	0

TABLE II

CHLORIDE CONTENT OF THE BLOOD IN SECONDARY ANEMIA

CASE NO.	RED BLOOD CELLS IN MILLIONS PER C.MM.	VOLUME OF RED BLOOD CELLS		VOLUME INDEX	CHLORIDE AS NaCl (MG. PER 100 C.C.)		DIAGNOSIS
		PER CENT OF WHOLE BLOOD	PER CENT OF NORMAL		PLASMA	WHOLE BLOOD	
1	1.03	9	19	0.91		560	Sepsis
2	1.69	15	31	0.91		540	Carcinoma of uterus
3	1.97	19	38.5	0.97	550	510	Malnutrition
4	2.01	20	42	1.00		520	Chr. nephritis
5	2.10	18	37	0.88		470	
6	2.16	22	45	1.04		540	Undetermined
7	2.18	21	43	1.00		490	Exhaust gas poisoning
8	2.26	22	46	1.00		530	Gastric hemorrhage
9	2.26	23	47	0.92		620	Banti's disease
	4.22	41	85	1.00		520	
10	2.95	27	56	1.20		470	Uterine hemorrhage
11	3.30	38	79	1.20		450	Hemolytic jaundice
12	3.40	28	58	0.87	570	520	Oral sepsis
	4.18	34	71	0.86	550	500	
13	3.42	31	65	0.96		490	Chr. cholecystitis
14	3.52	34	71	1.00		540	Pyelonephritis
15	3.56	36	75	1.05		470	Syphilis
16	3.69	29	61.5	0.83		470	Oral sepsis
	4.00	30	62.5	0.78		510	
	3.59	28	58	0.81	560	510	
17	3.78	37	77	1.02		470	Undetermined
	4.32	42	86	1.00		450	
18	3.78	32	68	0.90		470	Oral sepsis
19	3.79	36	75	0.99		466	Undetermined
20	4.65	29	62	0.67	610	570	Syphilis

These figures show that there is no constant variation in blood chlorides typical of anemia. It is still possible, however, that there may be a disturbance of chloride metabolism which is not reflected in the chloride level of the blood.

For comparison with the chloride content of the blood, I have noted in Table I, also the results of tests for free HCl acid in the gastric juice. None were present in any instance. There is no evident relation of the chloride level to the hydrochloric acid.

SUMMARY

Chloride determinations on thirty cases of pernicious anemia and twenty cases of secondary anemia are reported.

The whole blood chlorides are usually higher in blood with a low cell volume in both primary and secondary anemia.

The plasma chlorides remain about normal regardless of the cell volume.

A few cases of pernicious anemia show a low chloride content. No patients with secondary anemia had low chlorides.

There is no relation of the chloride level of the blood to the free hydrochloric acid of the gastric juice or to the volume index of the corpuscles.

The total store of chlorides in patients with very low cell volumes is higher than normal since the blood volume shows little variation from normal and the plasma chloride content is higher than whole blood.

These results give no positive evidence of a disturbance of chloride metabolism in pernicious anemia.

REFERENCES

[1]Levine, S. A. and Ladd, W. S.: Bull. Johns Hopkins Hosp., 1921, xxxii, 254.

[2]Cabot, R. C.: Osler's Modern Medicine, iv, 627, Lea and Febiger, Phila., 1908.

[3]Haden, R. L.: Jour. Am. Med. Assn., 1924, lxxxiii, 671.

[4]Meulgracht, E.: Arch. f. Verdauungskr., 1921, xxviii, 216.

[5]Haden, R. L. and Orr, T. G.: Jour. Exper. Med., 1923, xxxvii, 365 and xxxviii, sch. 55, Surg. Gyn. and Obst., 1923, xxxvii, 465.

[6]Emerson, C. P.: Deutsch. Arch. f. klin. Med., 1901, lxxii, 415.

[7]Haden, R. L. and Orr, T. G.: Bull. Johns Hopkins Hosp., 1924, xxxv, 58.

[8]Host, H. F.: JOUR. LAB. AND CLIN. MED., 1920, v, 713.

[9]Buckman, T. and Edwards H.: Jour. Am. Med. Assn., 1923, lxxx, 1644.

[10]Essen, H. and Porges, O.: Wien. Arch. f. inn. Med., 1922, v, 195.

[11]Christian, H. A.: Arch. Int. Med., 1916, xviii, 429.

[12]Gettler, A. O.: Jour. Am. Med. Assn, 1921, lxxvii, 1652.

LABORATORY METHODS

THE PAUL TEST IN THE DIAGNOSIS OF SMALLPOX*

By Charles E. Simon, M.D., and Joseph M. Scott, Sc.D.†

IN a paper published about a year ago, the writers drew attention to the value of the so-called Paul method in the diagnosis of smallpox.[4] Since then, the work has been continued, and it is the purpose of the present paper to emphasize the principal conclusion which was reached at that time, that, histologically controlled, the method is reliable and deserves general application in all doubtful cases. We would point out particularly that the material used in the examination, viz., the dried exudate from vesicles and pustules, retains its infectivity for some time and can hence be transported to the laboratory from long distances. We have examined specimens sent, not only from neighboring states, but from far away states like Colorado, and even from distant countries like China, and found that the material had not suffered in transit in the least.

Our studies during the past winter have further shown that, as Gins[2] pointed out, the smallpox virus may at times be demonstrable in the nasal secretion when, on the basis of the usual criteria of recovery, the individual would be ready for discharge from quarantine. It is accordingly suggested that a careful rhinoscopic examination be made in every case before release, and that this be supplemented by an examination of the swabbings, with the same method, if there is the least suggestion of an ulcerative lesion.

TECHNIC

Principle of the Method.—In principle, the method is based upon the demonstration of macroscopically visible lesions in the inoculated rabbit cornea, in the sublimate alcohol bath, and the appearance of the affected areas in sections. The latter phase of the examination is unnecessary if, as is the rule, the macroscopic examination gives a clear-cut picture. In our own work, however, the histologic control was carried out in every instance.

The technic, as here described, is essentially that of Paul,[3] with certain minor modifications which suggested themselves in the course of our work.

Preparation of the Material for Inoculation.—A few vesicles or pustules are cleansed with alcohol, wiped dry with sterile cotton and opened with a sterile needle or a small lancet. The exuding vesicular or pustular material is mounted on clean slides in the form of *thick* drops. These are allowed to

become air-dry and the specimens may then be wrapped in paper and sent to the laboratory in a suitable container. If the pustular stage has passed, a number of scales should be lifted from their beds and sent as such. Care should be taken, however, to secure only round, brown scales with a smooth surface. Yellow irregular crusts with fissured surfaces are usually devoid of virus. Serum from papules is not suitable for examination.

Inoculation of the Rabbit Cornea.—Both eyes of a rabbit are anesthetized with a few drops of a 5 per cent solution of cocaine hydrochloride, and then *delicately* scarified with a fine needle, making four or five horizontal and as many vertical scratches, about 1 mm. apart. A loopful of as heavy an emulsion in saline as can be prepared from the material submitted for examination, is then gently rubbed over the scarified surface of one eye. The other cornea serves for purposes of control. The animal is kept by itself in a cage, which is sterilized after use.

Examination of the Cornea in vivo—This should be done with a hand lense or a binocular loupe, using a good lateral illumination. Paul makes his final examination forty-eight hours after inoculation, and we have also done this in the majority of cases. In the presence of a positive reaction, however, a clear-cut picture can often be obtained after forty hours and if not *in vivo*, then certainly in the alcohol bath (see below). In examining the cornea, one should bear in mind that the smallpox virus does not produce *a keratitis by this time. One does not see any opacities*, but merely little dew-drop-like elevations which, in contradistinction to dust particles, with which they may be confused at times, are not movable when the eyelids are opened and closed. At this time already, some of the little blebs may present crater-like depressions.

Examination of the Enucleated Eyeball in Sublimate Alcohol.—In spite of the fact, that with experience a positive reaction may be recognized *in vivo*, we would recommend that the findings at this time, whatever they may be, be controlled by an examination in the alcohol bath. To this end the animal is killed by cervical shock and subsequently bled from the cervical vessels, so as to avoid bleeding during the enucleation of the eyeball. Both eyeballs are removed and immediately placed in a glass tray containing sublimate alcohol, taking care that the solution covers the eyes. The latter is composed of two volumes of a saturated solution of mercuric chloride and one volume of ordinary grain alcohol (94 to 96 per cent). The mercuric chloride solution is prepared by dissolving 4 grams of the salt in 60 c c of water, with the aid of heat, and then cooling and filtering. The examination should always be made against a black background and continued for from two to four minutes, by which time a maximum *differentiation* of the picture will have been obtained. At the expiration of that time the specimens are removed to 70 per cent alcohol, where the resulting appearances may be studied at leisure and photographed if desired.

Appearance of the Smallpox-infected Cornea and the Noninfected Cornea, after Treatment with Sublimate Alcohol.—In the sublimate alcohol bath, the control cornea becomes uniformly opaque, except for the lines of scarification

which occasionally are still visible. Not infrequently a slightly mottled appearance develops at first, but this gradually changes to a uniform opacity.

A smallpox-infected cornea presents an entirely different appearance. The general background here also is evenly opaque, but here and there within the area of scarification, and usually along the lines of the scratches and at
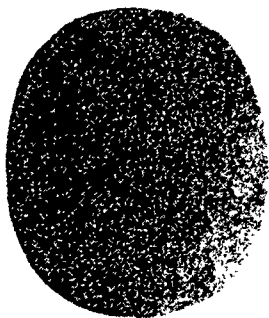
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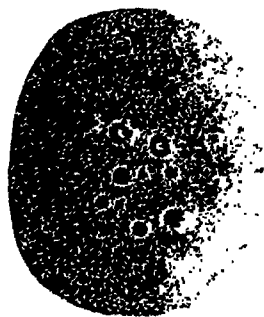
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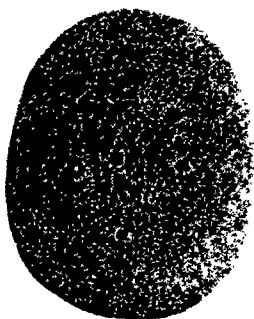
3

4

Plate I.

the points of intersection, *intensely white and opaque* little elevations make their appearance, some of which, even at this time, show crater-like depressions. These elevations correspond to the little dew-drop-like blebs which were seen *in vivo*. The number of elevations may vary from two or three to a dozen or more. They are usually discrete in outline, with clear-cut margins, though at times neighboring ones may be confluent. Not infrequently they occur in "string-of-pearl formation" along the lines of scarification.

Somewhat confusing pictures may present themselves when the variola virus is associated with large numbers of pyogenic organisms, as is so frequently the case during the suppurative stage of the disease. *In vivo* the appearance of the eye is different, inasmuch as corneal opacities of variable degree, with more or less extensive desquamation of the epithelium, control the picture and may obscure the focal changes due to the variola virus *per se*. There is then also more or less conjunctivitis with a corresponding exudation of pus. In the sublimate bath, however, it is almost always possible to reach a correct diagnosis, as some of the variolar blebs will be recognizable as such (Plate I, Fig. 3). When in doubt, of course, it is best to resort to the examination of sections. This should be done as a matter of routine and irrespective of the macroscopic findings, unless the observer has already acquired a considerable amount of experience. To this end Paul recommends the following procedure:

Histologic Technic (Rapid Method of Paul).—If the eyeball was examined in 70 per cent alcohol, after a preliminary sublimate alcohol bath of from two to four minutes, it is returned to the latter for approximately ten minutes. The cornea is then snipped out and placed in absolute alcohol for one minute. Then follows a series of baths, each of one minute's duration, in iodinized alcohol, absolute alcohol, a mixture of equal parts of absolute alcohol and chloroform, chloroform, a saturated solution of hard paraffin in chloroform, at 40° C., and liquefied hard paraffin at 60° C. The cornea is now embedded in the usual manner, with its convex side either up or down. The block, after being permitted to harden in ice water, is cut through at right angles to the cornea; the two halves are up-ended, mounted and sectioned, the idea of division and up-ending of the cornea being to obtain sections passing through the lesions, as soon as possible. Serial sections are mounted in the usual manner, "blotted" with several layers of lense paper that has been well moistened with absolute alcohol, and placed in the drying oven at 60° C. for a few minutes. The sections are next deparaffinized in xylol, run down through the alcohols, stained for five minutes in hemalum, run through the alcohols upwards, cleared in xylol, and mounted in balsam. This is the technic which Paul recommends. In practice we have not obtained satisfactory results in this manner, and prefer to *proceed a little more leisurely*, particularly with the processes of dehydration and infiltration with paraffin. The allowance of more time will give more satisfactory pictures and do away with the necessity of redehydration and reembedding which overhaste only too often requires.

The Histologic Picture of the Variola-infected Cornea.—In sagittal sections the most striking features are the epithelial hummocks (Plate II, Fig. 1) which correspond to the little protuberances that one sees in the sublimate alcohol bath or in the living eye. These hummocks, at this stage of the process, are the result of an increase in the size of the epithelial cells, due to the imbibition of fluid. The basal cells become involved first, and in consequence of their increase in both length and breadth, they are thrown out of their normal position in reference to one another, as well as to the overlying cells, which then also undergo hydropic changes. A picture thus results which
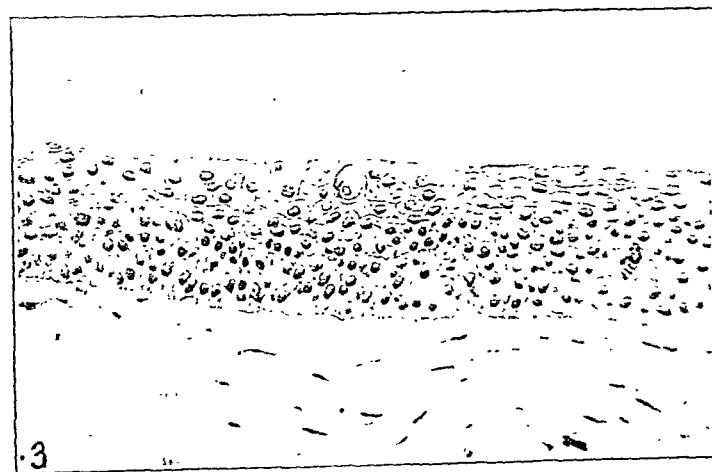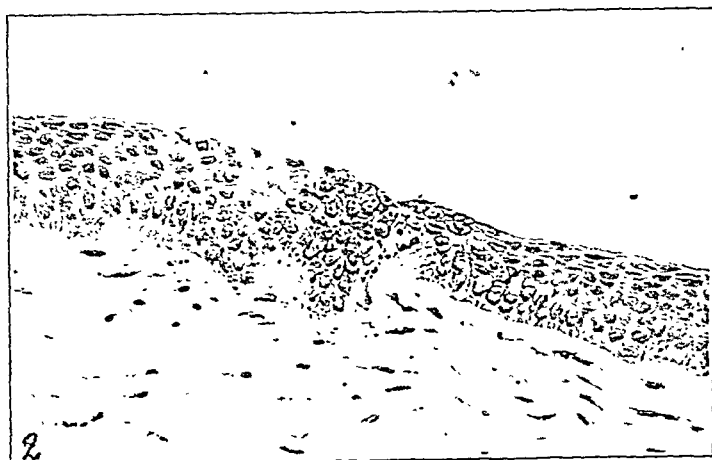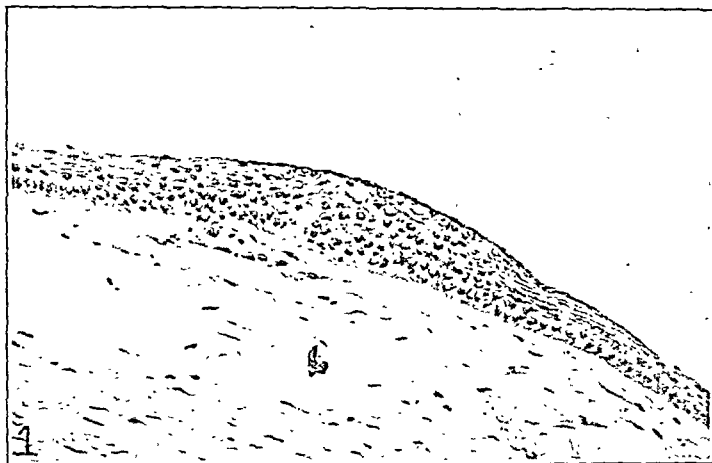
Plate II.

suggests a far more extensive degree of stratification of the cells than is normal for the cornea. The breadth of the epithelial layer in the center of the hummock is almost twice that of the normal peripheral zone. The pathologic process evidently extends peripherally, for the most advanced changes are encountered in the center of the hummock, and it is in this portion that desquamative changes first take place, with the consequent production of crater-like defects. With beginning central desquamation so-called box cells (Schachtelzellen) frequently appear in this area (Plate II, Fig. 3), and it is here also that the Guarnieri bodies are most numerous (Plate II, Fig. 2). The cells in question were first described by Hückel (1898) and named Schachtelzellen to denote their supposed origin, one cell becoming boxed up in another, as the result of pressure invagination, and the pair in turn in yet another, and so on.

The Guarnieri bodies, as seen in sections, are of variable size, ranging from a micron to about one-third the size of the nucleus of the epithelial cell. They are usually round, oval or lenticular, but not infrequently a little irregular in form. It is generally stated that they stain with nuclear dyes. This is true, when nuclear dyes only are used, but when a mixture of a nuclear dye and an acid dye is employed, such as eosin and hematoxylin, they present a mixed tone, in which the affinity for the acid dye seems to be more pronounced. Paul lays special stress on the prime importance of the character of the epithelial lesions, and regards the presence or absence of the Guarnieri bodies as of secondary value, from a diagnostic standpoint. He emphasizes also that the search for them may be a tedious one, and that they appear only in larger numbers at a relatively late stage. We are quite willing to admit the specificity of the epithelial changes and their diagnostic value, when they are clear-cut, but it may happen that these changes are not sufficiently pronounced to serve alone as criteria for diagnosis In such cases the search for Guarnieri bodies is imperative, and their presence must be viewed as deciding evidence in favor of a positive diagnosis. In searching for them it should be borne in mind that they occur only in the diseased areas, viz., in areas where the normal arrangement of the cells has evidently been upset. It is true that these bodies appear in larger numbers later in the development of the lesions, but there can be no question that at the time when the animal is usually killed for the Paul test, i.e., forty-eight hours following inoculation, they are not so scarce that they are difficult to find

Personal Observations.—In our first paper[4] we reported our findings in the course of the examination of 104 corneas. Since then the number of individual examinations has reached 247. The series now comprises forty-five examinations following inoculation with pustular material from active cases of smallpox; twelve examinations following inoculation with nasopharyngeal swabbings from smallpox convalescents; twenty-seven inoculations with vesicular fluid and eleven with nasopharyngeal swabbings from cases of chickenpox; sixty-seven normal controls of which nineteen had not even been scarified. In addition, corresponding examinations were made in twelve cases of herpes simplex; nine of herpes zoster; two of herpes genitalis; five of pem-

phigus; one each of dermatitis herpetiformis and ringworm, besides a number of examinations after inoculation with streptococci and staphylococci.

Our results bear out the validity of Paul's claim that the method is a dependable one and well suited for purposes of diagnosis.

In forty-four of the forty-five cases of bona fide smallpox the macroscopic reaction was in itself sufficient to reach a diagnosis. In only one was the result noted as "doubtful," but in this instance, as in all others in which sections were examined (thirty-nine), the histologic picture was a clear-cut positive for smallpox. In none of the varicella cases or any of the various skin affections which were examined was a reaction noted that even remotely was suggestive of a positive reaction. The histologic picture in these cases is, of course, also very different. In no instance were epithelial hummocks noted or any cell inclusions which in the least resembled the Guarnieri bodies. In the case of herpes simplex, herpes genitalis, dermatitis herpetiformis and pemphigus, there were markedly destructive lesions, with extensive epithelial desquamation, while in herpes zoster neither a conjunctivitis nor a keratitis was observed; in none of the nine cases of herpes zoster, moreover, were cell inclusions of any kind encountered.

PERSISTENCE OF THE SMALLPOX VIRUS IN THE NASOPHARYNX OF CONVALESCENTS

Friedmann and Gins[2] have pointed out that with the Paul method, the virus of smallpox may at times be demonstrated in the nasopharyngeal secretion of active cases and convalescents. According to their findings, the virus may indeed persist in the nasopharynx for several weeks.

During the past winter, through the courtesy of John Collinson of the Maryland State Board of Health, we had the opportunity to examine two cases of smallpox at the termination of the active stage of the malady and again at the time of the usual release from quarantine. In the one instance, a positive Paul reaction was obtained with the nasopharyngeal swabbings on the thirty-first day of the disease, and in the other on the twenty-third day, at which time the skin lesions had entirely disappeared.[5]

Control examinations with material from two normal persons and from nine cases of chickenpox in various stages of the disease, as well as from two chickenpox contacts, gave negative results. In three additional cases of smallpox, in which the swabbings had been taken respectively on the eighth, ninth and eighteenth days of the disease, the results were negative. It is noteworthy that in the two positive cases the process of swabbing was quite painful, while the three negative patients made no complaint, which suggests that in the first case two ulcerative processes were still going on.

CONCLUSIONS

1. The so-called Paul reaction, when controlled by histologic examination, constitutes a most valuable method for the diagnosis of smallpox.

2. In many cases the macroscopic picture of the inoculated rabbit cornea, in the sublimate alcohol bath, is in itself sufficient for diagnosis.

3. A negative macroscopic result will probably always warrant a negative diagnosis as to smallpox, if satisfactory material is available for the examination.

4. As in other diagnostic laboratory tests, the validity of the results will depend in part upon the experience of the worker and the technic employed in procuring the material for examination.

5. In chickenpox, negative results are so constant that a doubtful reaction should arouse suspicion and call for a further examination.

6. Pyogenic infections of the cornea *per se* do not interfere with the reaction.

7. The smallpox virus was found in one instance to retain its activity in the dried-drop preparation, at ice box temperature, for 387 days.

8. Before release from quarantine a careful rhinoscopic examination should be made in all smallpox cases, and followed, if need be, by the application of the Paul test to the nasopharyngeal secretion.

REFERENCES

[1] Gins, H. A.: Erfahrungen mit d. experimentellen Pockendiagnose nach Paul, Deutsch. med. Wchnschr., 1916, p. 1118.

[2] Gins, H. A., and Friedmann, U.: Experimentelle Untersuchungen ueber die Uebertragung der Pocken, Deutsch. med. Wchnschr., 1917, No. 37.

[3] Paul, G.: 1915. Zur Differentialdiagnose d. Variola und d. Varizellen. Centralbl. f. Bakteriol., Part I, Orig. 75, p. 523.
   1916. Ueber Biologie u. Histologie d. specifischen Epithelveraenderungen auf d. variolierten Kornea d. Kaninchenauges. Ber. ueber d. 40. Versamml. d. ophthalmol. Ges., Heidelberg.
   1916. Objektive Sicherung d. Varioldiagnose durch d. Tierversuch. Wien. med. Wchnschr., No. 23, p. 861.
   1916. Zur Variolaprobe. Der Amtsarzt, Nos. 4-6, p. 129.
   1917. Zur histologischen Technik d. Kornealversuches bei d. Pockendiagnose. Deutsch. med. Wchnschr., No. 29, p. 900.
   1917. Entwicklungsgang d. Pockenepitheliose auf. d. geimpften Kaninchenhornhaut. Deutsch. med. Wchnschr., No. 45, p. 1415.
   1918. Ueber Mischinfektionen auf. d. geimpften Kaninchenhornhaut. Centralbl. f. Bakteriol., Part 1, Orig. 80, p. 361.
   1919. Aetiologische Untersuchungen bei Variola. Uebersichtsreferat. Beitr. z. Klin. d. Infektionskr., VII, 267-290.

[4] Scott, J. M. and Simon, C. E.: The Diagnosis of smallpox by the Paul method. Am. Jour. Hyg., 1923, iii, 401-415.

[5] Scott, J. M.: The Release of the Smallpox Patient from Quarantine. Am. Jour. Hyg., 1924, iv, 152.

AN IMPROVED COLORIMETER*

By John A. Killian, Ph.D., New York City

THE rapid development of colorimetric methods within the past decade testifies to the practicability of these quantitative procedures in biochemistry; however, their general simplicity may tempt the unwary to sacrifice accuracy for speed. That the analyst should be familiar with all the fundamental chemical reactions upon which the analytical methods have been based, has frequently been emphasized. Moreover, it is equally essential for maximum accuracy that the colorimetry be carried out under standard conditions, particularly of light. This need presents a problem in the average hospital and students' laboratories. The qualities of daylight are too variable and artificial daylight lamps are both cumbersome and costly, and the source of light is not fixed in relation to the solutions being matched. The improvements introduced in the colorimeter described, we find, satisfy this need in our everyday work. The instrument is not entirely new, it has been constructed by utilizing the best mechanical parts of the Klett and Bock-Benedict colorimeters, with a few additional improvements.

The general appearance of the colorimeter is shown in Diagram I. It is a plunger type of colorimeter, weighing about 2.3 kilograms and standing 30 cm. high. The instrument occupies but a small amount of space and may be read with comfort on a laboratory desk of average height. The cups are of black glass with overflow tops and fused on clear glass bottoms. Similarly the cylindrical plungers are of black glass with clear glass bottoms fused on. In this union of the clear glass to the black glass, simple fusion without a flux has been employed. The repeated use of mixtures of chloroform, acetic anhydride and sulphuric acid, such as are necessary in cholesterol estimations, have not injured the cups or plungers. The diameter of the plungers is 1.1 cm., that of the cups 1.4 cm., and but 4 c.c. of fluid are required to fill the cups conveniently for reading.

The cups are supported on mounted stages by rack and pinion on separate nickel-plated pillars. The rack and pinion are constructed of brass and operate on a spring system. This mechanism has been found, in the Bock-Benedict colorimeter, to minimize the lost motion due to wear after continued use. The light from the plungers is reflected into the eyepiece by Helmholtz prisms. On the posterior portion of the prism housing, a mirror placed at an angle of 45° facilitates the reading of the vernier scale. The zero point of the scale on both pillars is readily adjustable by a thumb screw. The reading mirror and vernier scale are similar to those of the Klett colorimeter. The base measures 14 by 11 cm. and is relatively heavy so that the instrument is un-

usually well balanced. On the top of the base there is a ground glass mirror for use in daylight. One border is fixed to the base and the mirror may be moved through an arc of 90° and firmly secured at any position by an adjustable screw. Turning this mirror forward at a right angle with the base discloses a built-in substage lamp for artificial daylight. This substage lamp is composed of a glass tubular electric bulb with a frosted surface. A long slender filament extends around the entire periphery assuring a uniform intensity of light from the entire surface area of the bulb. The electric bulb (25 Watt) is covered by a blue glass of good neutral color. The fact that both cups are receiving equal intensities of light is ensured by the fixed
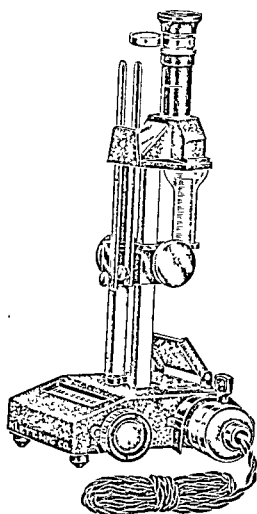
Diagram I.

position of the daylight lamp in relation to the cups, which renders this instrument far superior to any other colorimeter with artificial daylight. In fact it has been found that the color comparisons are more uniform with the substage lamp than with the varying qualities of daylight in the average laboratory. The lamp is readily turned on or off by means of a switch at the right-hand side of the base. It appeared possible that the temperature of the solutions in the cups might be influenced by the use of this lamp, but there was noted no rise in temperature of solutions in the cups during a period of ten minutes continuous illumination with the lamp. There is no necessity, however, for using the lamp except during the period of color comparison.

The posterior portion of the base carries tables facilitating the calculation of results. The tables are printed in black on a linen sheet, which is

operated by a hand wheel. Switching on the substage lamp also illuminates the tables. Since all color comparisons are made with the standard set either at 15 or 20 mm., the calculations for $\frac{15}{R}$ and $\frac{20}{R}$ have been tabulated. The recorded results are for readings for the unknown between 6 and 37, and have been calculated to the first decimal place. The method of making a reading and of utilizing the table of calculations is illustrated in Diagram II which gives the actual size of figures. In any series of calculations, the two unknown factors are the reading and the dilution. The strength of
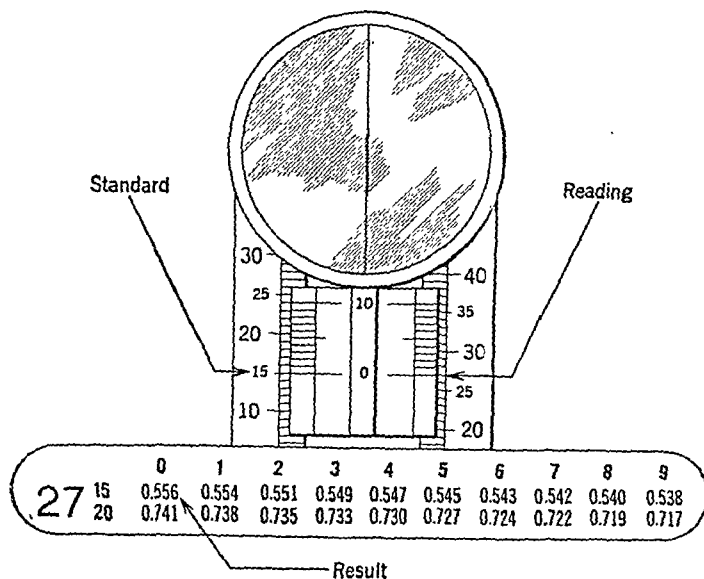
Diagram II.

the standard is a constant. Every equation will have this general form $\frac{15 \ (or \ 20)}{R} \times s \times dil = result$. The result of the fraction $\frac{15 \ (or \ 20)}{R}$ is determined immediately from the table. For example, if the standard has been placed at 15, and the reading is 27, reference to the table shows that $\frac{15}{27}$ is 0.556. The figures from 0 to 9, across the top of each section of the table represent the first figures to the right of the decimal point in the reading. Although the table presents calculations for readings from 6 to 37, to insure accurary, readings should not be made below 10 nor above 20 when the standard has been set at 15, and not below 15 nor above 25 when the standard has been set at 20.

A separate table is provided for the calculations of the hemoglobin of the blood. In the method described by Myers 0.05 c.c. of blood are added to 20 c.c. of 0.1 N HCl and thoroughly mixed. After standing at least four hours, the hematin hydrochloride thus formed is compared with a standard disc. In the colorimeter described the disc is fitted into a brass shoulder.

The plunger on the left hand side is unscrewed from the prism housing, and the disc with its shoulder fits into the space provided in the brass cap at the top of the plunger. The plunger is then screwed back into place. The cup on the left is about three-quarters filled with water and set at 10. The unknown on the right hand side is compared with this standard The reading is noted, and a reference to the table gives the grams of hemoglobin in 100 c.c. of blood corresponding to the reading. For this result the following formula has been used: $\frac{10}{R}$ x 0.38 x 400 = per cent of hemoglobin The intensity of color in the disc corresponds to 0.038 per cent of hemoglobin in 0.1 N HCl placed at 10 in the colorimeter.* For example, if the unknown read 10.5, it is evident from the table that the specimen contains 15.2 grams of hemoglobin per 100 c.c., i.e., 15.2 per cent. For the proper interpretations of the results of blood analysis, an accurate knowledge of the hemoglobin concentration is indispensable. If the hemoglobin is unusually low 0.1 c.c. of blood may be used in place of 0.05 c.c., and a correction is made by dividing the final result by 2. When the hemoglobin disc is not in use, it is securely and conveniently held in a small brass cup on the base.

The telescopic eyepiece has one lens, permitting an adjustment of the focus through a wide range. The small aperture in the diaphragm of the eyepiece limits a well-defined field, as illustrated in Diagram II. When the dividing line between the two halves of the field is brought into sharp focus, the periphery of the field is also sharply focussed. On the posterior portion of the eyepiece there is attached a bifocal lens; the anterior half of this lens magnifies the image of the vernier scale in the reading mirror, the posterior half magnifies the calculation tables on the base of the colorimeter.

The colorimeter described presents some points of superiority over the instruments now in use. The substage lamp fixed in the base renders it possible to use the colorimeter anywhere and at any time in the laboratory. It is small, compact, and, hence, occupies little space. The calculation tables are an invaluable aid in a long series of calculations. The lenses permit reading the vernier and tables with accuracy and comfort.

<div align="center">REFERENCES</div>

1Myers, V. C.: Practical Chemical Analysis of Blood, ed. 2, St. Louis, 1924, C. V. Mosby Co.
    The appendix of this book gives an excellent description of the various types of colorimeters and their use.

*This disc has been calibrated against hemoglobin of blood specimens determined by the oxygen capacity method.

SIMPLIFIED COLORIMETRIC CALCULATION[*]

BY WILLIAM H. STONER, A.M., PHAR.D., M.D., PHILADELPHIA, PA.

FOR the past eight years these laboratories have employed a simplified method of colorimetric calculation which, at the time of its introduction, seemed too obvious to justify its publication. Since that time, however, so many cumbersome tables,[1, 2, 3] graphs,[4] nomographs, special slide rules, etc., have been devised, complicating a most simple arithmetic process, that it has been suggested that the simple manipulatory artifice for avoiding routine colorimetric calculation be made known. Casual mention of the method has been published.[5] No doubt many laboratories are using an identical method, but, judging from many recent articles and textbooks, there are many workers in colorimetry who could reduce their routine calculations by the use of this method.

Just as factor solutions are employed to eliminate calculation in routine volumetric analysis, so can factor settings of the colorimeter be so chosen as to make the results of routine determinations derivable by inspection. Thus, the calculation of a routine determination may be made initially, once and for all, not only saving time but also entirely precluding the error inherent in the chemical technician's arithmetic.

The usual recommendation[6-9] for colorimetric work is to set the standard solution (in the left cup for a right-handed operator) at some fixed point, as 10 or 15 mm. depth, and to adjust the unknown solution until color equality is obtained. This necessitates division by the reading of the unknown. A simple reversal of conditions eliminates this division. Instead of fixing the standard solution at a definite reading and adjusting the unknown solution to color equality, the standard solution (in the right cup for a right-handed operator) is adjusted to match the unknown solution set at some fixed point so calculated that the reading of the standard becomes the sought result of the determination or some simple multiple or submultiple thereof.

Probably the most useful and well-known field for exemplification of this manipulatory evasion of routine calculations is that of clinical blood chemistry. Similar methods may be applied to all colorimetric determinations.

Following is the method of determining a factor colorimetric depth of solution derived from blood so as to obviate paper and pencil, nomographic, logarithmic, slide rule or tabular routine calculations. The method is here applied to the Folin and Wu[10] determinations of dextrose, total nonprotein nitrogen and creatinine, to the Benedict[11] uric acid determination, to the Myers and Wordell[12] cholesterol determination and to the Briggs[13] determination of inorganic phosphorus. A general method is given in order that sim-

ilar reasoning may be applied readily to all other colorimetric determinations, as has been done in these laboratories. Letters have been assigned the various values merely that the demonstrations for the six determinations may be shown compactly in tabular form.

Let $x =$ mg. of sought constituent in 100 c.c. of blood (the conventional clinical method of expression of results of chemical blood analyses).

$b =$ reading (in mm.) on colorimeter of solution derived from blood.

$s =$ reading (in mm.) on colorimeter of solution containing standard.

$V =$ total c.c. of colorimetric solution containing standard.

$v =$ total c.c. of colorimetric solution derived from blood.

$C =$ mg. of sought constituent (standard substance) in v c.c. of the colorimetric solution containing standard.

$c =$ mg. of sought constituent in the total volume of colorimetric solution derived from blood.

$B =$ total c.c. of blood sample represented by the colorimetric solution derived from blood.

Since $c =$ mg. of sought constituent in B c.c. of blood, the mg. of sought constituent in 100 c.c. of blood, or

$$x = \frac{100\ c}{B} - - - - - - - - - (1)$$

From the universal colorimetric proportion (Beer's law).

$$C : c = b : s \text{ or}$$

$$c = \frac{C\ s}{b}$$

Substituting this value of c in (1),

$$x = \frac{100\ Cs}{B\ b}$$

Let

$$K = \frac{100\ C}{B}$$

When

$$x = \frac{K\ s}{b}$$

The value of K in each particular analysis determines the most convenient setting of the colorimeter scale for the colorimetric solution derived from blood.

Table I.—From the values of x, in terms of the readings of the adjusted standard solution, in the last column of the table it is apparent that the milligrams of constituent per 100 c.c. of blood may be read from the colorimeter scale either directly as in the case with nonprotein nitrogen, or by such simple processes as changing the position of a decimal, or multiplication or division by 2, 5, etc.

It has been suggested by Walter G. Karr and Bernard L. Oser, whose experience in the instruction of internes and technicians is large, that this method would be more useful and more easily understood if literal values for the various quantities especially of K, were avoided. Accordingly they use the practical teaching formula given on page 576

It is seen that the mg. of constituent per 100 c.c. blood is the product of four factors, designated for brevity, (1) the colorimetric factor, (2) the standard factor, (3) the sample factor and (4) the dilution factor. When the

final colorimetric solutions are equal in volume, the dilution factor, (4) is (1) and may be ignored.

The values of the terms of this formula given in the original methods are modified in some cases, cholesterol for example, in these laboratories for

$$\left(\begin{array}{c}\text{Mg. of constituent}\\\text{per 100 c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard}}{\text{Reading of unknown}}\right) \times \left(\begin{array}{c}\text{Mg. of standard}\\\text{in colorimetric}\\\text{solution}\end{array}\right) \times \frac{100}{\left(\begin{array}{c}\text{C.c. of blood used}\\\text{for colorimetric}\\\text{solution}\end{array}\right)} \times \left(\frac{\text{C.c. of unknown colorimetric solution}}{\text{C.c. of standard colorimetric solution}}\right)$$

$$\left(\begin{array}{c}\text{Mg. of dextrose}\\\text{per 100 c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard No. 1}}{20}\right) \times 0.2 \times \frac{100}{0.2} \times \frac{25}{25} = 5 \times \text{Reading of standard No. 1.}$$

$$\left(\begin{array}{c}\text{Mg. of nonprotein}\\\text{nitrogen per 100}\\\text{c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard}}{30}\right) \times 0.3 \times \frac{100}{0.5} \times \frac{50}{100} = \text{Reading of standard.}$$

$$\left(\begin{array}{c}\text{Mg. of Creatinine}\\\text{per 100 c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard}}{30}\right) \times 0.03 \times \frac{100}{1} \times \frac{15}{30} = 0.05 \times \text{Reading of standard.}$$

$$\left(\begin{array}{c}\text{Mg. of uric acid}\\\text{per 100 c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard}}{20}\right) \times 0.02 \times \frac{100}{0.5} \times \frac{15}{15} = 0.2 \times \text{Reading of standard No. 1.}$$

$$\left(\begin{array}{c}\text{Mg. of cholesterol}\\\text{per 100 c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard}}{16}\right) \times 0.4 \times \frac{100}{0.25} \times \frac{7.1}{7.1} = 10 \times \text{Reading of standard.}$$

$$\left(\begin{array}{c}\text{Mg. of phosphorus}\\\text{per 100 c.c. blood.}\end{array}\right) = \left(\frac{\text{Reading of standard}}{12.5}\right) \times 0.05 \times \frac{100}{1} \times \frac{9}{18} = 0.2 \times \text{Reading of standard.}$$

As an aid to the memory for rapid routine work Table II is given.

the purpose of deriving more convenient factor colorimetric settings. To avoid confusion of the method, however, in the following formulas the original values are retained.

TABLE I

NUMERICAL DATA FOR CALCULATING SIMPLIFIED FACTOR FORMULAS FOR THE COLORIMETRIC DETERMINATIONS OF CHEMICAL CONSTITUENTS OF BLOOD

CONSTITUENTS	F	v	C		B	K		x		b		x	
			STANDARD 1	STANDARD 2		STANDARD 1	STANDARD 2	STANDARD 1	STANDARD 2	SETTING 1	SETTING 2	SETTING 1	SETTING 2
Dextrose	25	25	0.2	0.4	0.2	100	200	$\frac{100\,s_1}{b}$	$\frac{200\,s_2}{b}$	10	20	$\frac{10\,s_1}{20\,s_2}$	$\frac{5\,s_1}{10\,s_2}$
Nonprotein Nitrogen	100	50	0.15		0.5	30		$\frac{30\,s}{b}$		30	15	8 s	2 s
Creatinine	30	15	0.015		1	1.5		$\frac{1.5\,s}{b}$		30		0.05 s	
Uric Acid	15	15	0.02	0.05	0.5	4	10	$\frac{4\,s_1}{b}$	$\frac{10\,s_2}{b}$	10	20	$\frac{0.4\,s_1}{s_2}$	$\frac{0.2\,s_1}{0.5\,s_2}$
Cholesterol	7.1	7.1	0.4		0.25	160		$\frac{160s}{b}$		16		10	
Phosphorus	18	9	0.025		1	2.5		$\frac{2.5s}{b}$		12.5		0.2	

TABLE II

FACTOR SETTINGS OF COLORIMETRIC SOLUTIONS DERIVED FROM BLOOD, TOGETHER WITH THE CORRESPONDING NUMBERS BY WHICH THE READINGS OF THE STANDARDS ARE MULTIPLIED TO OBTAIN THE MG. OF THE RESPECTIVE CONSTITUENTS PER 100 C.C. OF BLOOD

CONSTITUENT	DEXTROSE	NONPROTEIN NITROGEN	CREATININE	URIC ACID	CHOLESTEROL	PHOSPHORUS
Set unknown at	20	30	30	20	16	12.5
Multiply reading of standard (#1) by	5	1	0.05	0.2	10	0.2

SUMMARY

The simple, and universally applicable, manipulatory method of obviating routine colorimetric calculations with their accompanying errors by setting the unknown colorimetric solution at a fixed depth and adjusting the standard solution to color equality is illustrated by six examples in blood chemistry.

REFERENCES

[1]Gradwohl, R. B. H., and Blaivas, A. J.: Blood and Urine Chemistry, Ed. 2, St. Louis, 1922.
[2]Haden, R. L.: Clinical Laboratory Methods, St. Louis, 1923.
[3]Falisi, J. Vincent, and Lawton, Vera A.: JOUR. LAB. AND CLIN. MED., 1924, ix, 329.
[4]McDanell, Louise: JOUR. LAB. AND CLIN. MED., 1916, i, 804.
[5]Karr, Walter G.: JOUR. LAB. AND CLIN. MED., 1924, ix, 329.
[6]Gauss, Harry: JOUR. LAB. AND CLIN. MED., 1920, vi, 699.
[7]Shaffer, Philip A.: Jour. Biol. Chem., 1914, xix, 285.
[8]Stone, Willard J.: Blood Chemistry Colorimetric Methods, New York, 1923.
[9]Myers, Victor Caryl: Practical Chemical Analysis of Blood, Ed. 2, St. Louis, 1924.
[10]Folin, Otto and Wu, Hsien: Jour. Biol. Chem., 1919, xxxviii, 81.
[11]Benedict, Stanley R.: Jour. Biol. Chem., 1922, li, 187.
[12]Myers, Victor C., and Wordell, Emma L.: Jour. Biol. Chem., 1918, xxxvi, 147.
[13]Briggs, A. P.: Jour. Biol. Chem., 1922, liii, 13.

THE DETERMINATION OF GLOBULIN INCREASE IN SPINAL FLUID*

BY GEORGE E. ROCKWELL, M.D., M.A., CINCINNATI, OHIO

THE tests in general use for globulin increase in spinal fluid are: Noguchi's butyric acid method, the stratification with saturated ammonium sulphate solution, or Pandy's carbolic acid method. Noguchi's method is by far the better, but it is objectionable because of the butyric acid odor. The ring test with saturated ammonium sulphate, is too slow in appearing to give complete satisfaction, while Pandy's carbolic acid method does not give detailed information about the amount of increase of globulin, and the border line between the normal amount and increased amount, is not clear-cut.

I have found that the following solution which is a modification of Robert's solution (used for testing urine for albumin), is of very great value in the testing of spinal fluid for increased globulin. The solution consists of one part concentrated nitric acid, four parts of water which has been saturated with both magnesium sulphate and sodium chloride.

The test is performed by adding in a small test tube, one-half to one cubic centimeter of spinal fluid; then one cubic centimeter of the reagent is introduced with a pipette, placing the pipette at the bottom of the tube and allowing the reagent to run in under the spinal fluid. A definite line of demarkation can be seen between the reagent and the spinal fluid and if there is any increase in globulin, a cloudiness will immediately appear in the form of a ring. This cloudy ring can be broadened by gently agitating the tube. The amount of increase is indicated by the size and intensity of the ring.

The value of this test for increased globulin in the spinal fluid over the older methods is that there is no objectionable odor, it reacts quicker and better than ammonium sulphate test and the line of demarkation between a normal amount and an increased amount of globulin, is clear-cut.

A DRYING APPARATUS FOR LABORATORY VESSELS*

By Joseph C. Bock, Ch.E., Ph.D., and Max Gilbert, Ph.D., Milwaukee, Wis.

THE most widely adopted method for drying the inside of glass vessels consists in rinsing with strong ethyl alcohol, and removing the residual alcohol by means of ether. An alternative is the drying in an air oven. The alcohol-ether procedure, while fairly rapid, is rather costly. Besides that, vessels so dried, usually do not drain perfectly, evidently on account of some slight impurities in the ether. Furthermore, it must be considered that pure ethyl alcohol cannot be used as freely as it was used a few years ago. The drying in an oven is a rather time-consuming process.

It is the purpose of the present paper to describe an easily constructed, inexpensive drying apparatus. The arrangement as described has been used in this laboratory for over two months with very satisfactory results, and has been found superior to the above mentioned methods in every way.

The apparatus requires for its use compressed air and a Bunsen burner. The principle is simple. A stream of air is passed through a coil which is heated by a gas flame. The heated air escapes through one or several perforated tubes over which the vessels to be dried are suspended.

The dryer is made of standard one-eighth inch pipe and fittings which may be procured at any plumbing supply house. The construction is herewith described:

The heating coil is made first. This is done by joining two one-eighth inch, ninety degree elbows (technically termed "Ells") by means of a close nipple. Into one end of this U-shaped arrangement, another close nipple is inserted. On to this is screwed another elbow, then another close nipple, continuing this way alternately until the spiral has the desired length.

In the lower tiers of the coil the elbows are not brought together very closely, the distance between the flanges being about one-half inch. In the upper tiers the elbows are brought together more closely by giving a few. more turns, so that the sides of the coil taper towards the top. The number of elbows used is best given by referring to the illustration. The left front corner has eight elbows, the right front corner seven, the left rear corner seven, and the right rear corner the same number.

Into the lowest elbow of the coil (seen at the lower left front corner), is screwed a four and one-half or five inch nipple; this is joined by means of a union to another four or five inch nipple. The free end of this is connected with the compressed air supply by a rubber tube.

To the last elbow on top is attached a U, made of two elbows and a close nipple, as illustrated. A six inch nipple is attached to this, then follows
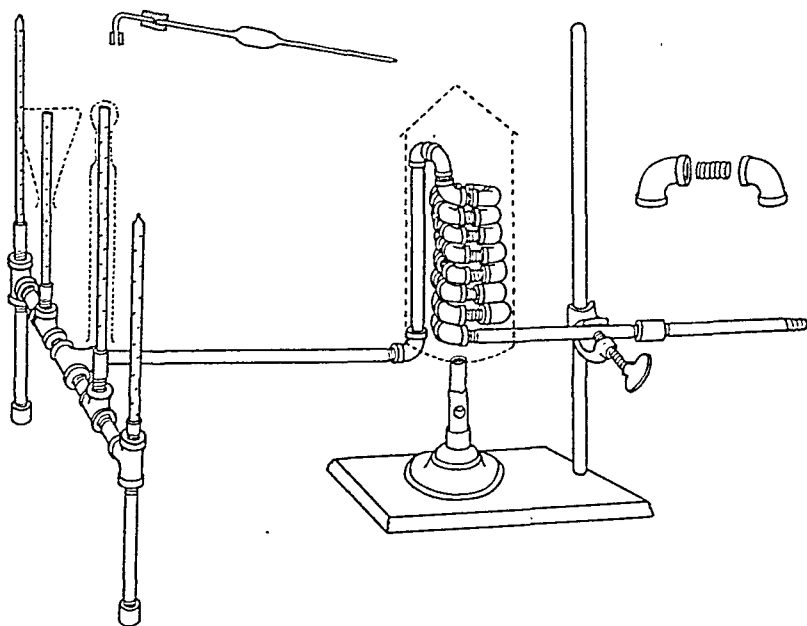
Fig. 1.

another elbow and another six inch nipple. Screw on to this a T. Into the free ends of the T are inserted short nipples (the short nipples are somewhat longer than the close nipples). There follows on each side a T, a short nipple, and another T. This last T is attached to the nipple by the middle opening. Three inch nipples, closed by caps, form the legs. The third point of support is a small ring stand, to which the intake pipe is held by an ordinary clamp holder.

Into the free openings of the four T's the drying tubes are inserted. These are made as follows:

A piece of one-eighth inch brass tubing is driven into a close nipple, the outside diameter of the brass tubing being slightly larger than the bore of the nipple. When doing this, the nipple should rest on a piece of hard wood, not on metal, otherwise the thread will be spoiled. It may happen that

the brass tube has a rather loose fit. In this case, the upper end of the nipple is pinched in a vise, thereby tightening on the tube. If an air-tight union is desired (desirable but not imperative), a little solder should be applied around the upper end of the nipple. The brass tubes may be of varying length, depending on the vessels that are usually dried. We prefer tubes about nine inches long. When only small tubes and flasks are to be dried, shorter tubes are advisable. Several holes are drilled into the tubes allowing the heated air to come in contact with the walls of the vessel in several places. To obtain the best results, these holes are made with a very small drill (size 54-60), the holes being drilled straight through, each pair of holes about 10 mm. distant from the next. The line of one pair of holes is at a ninety degree angle to the next. The opening on top is narrowed to a small slit by pinching the end of the tube in a vise or clamp. The drying tubes are screwed into the openings of the T's by hand and gently tightened with pliers. They should not be screwed in too tight, otherwise it may be found very difficult to remove them later on.

For heating, we use a Bunsen burner sawed off to fit under the coil. The coil is covered with a short piece of small stove pipe, or by wrapping asbestos paper around it, holding the asbestos in place with wire. The top of the covering is closed in a suitable way to prevent undue loss of heat.

When using the dryer, the coil is first heated for a few minutes with the air turned on. The apparatus is now ready for use. The vessels to be dried are hung over the tubes. When short test tubes or small flasks are dried, the lower part of the drying tubes may be closed, if desired, by slipping a loose fitting sleeve over it. This sleeve is made from a piece of hollow curtain rod of appropriate size.

For the drying of pipettes the following arrangement has been found effective. A piece of the brass tubing, about three inches long, is joined to a nipple in the manner described above. The upper end of the tube is now heated to a red heat, the end, which is attached to the nipple, being held in a piece of wet cloth. The tube may be bent very easily after it is cold. It is bent over until the end points slightly downward. The tube is screwed into one of the openings. A hole large enough to accommodate the pipe is bored in a cork, the cork is slipped half way into the drying tube. Into the other end the pipette is inserted, the tip of the pipette resting on a piece or pad of filter paper on the table on which the dryer stands.

When drying tubes of very small diameter, or larger tubes with constrictions, such as Folin-Wu sugar tubes, it may be necessary to decrease the outside diameter of the drying tube by filing, or better, by turning down with a lathe. The drying of a small flask or a large tube, such as a Folin-Wu sugar tube, is usually accomplished in a minute or less. Smaller tubes are dried in about thirty seconds.

The apparatus can be made for less than four dollars, if a drill press is available, otherwise the cost for drilling the small holes in the drying tubes has to be added.

SUMMARY

An inexpensive, easily constructed apparatus for the drying of glass vessels by heated air is described.

A NOTE UPON A METHOD FOR THE PREPARATION OF ANTISHEEP HEMOLYSIN*

BY ROBERT A. KILDUFFE, A.B., A.M., M.D.

IN a previous communication[1] concerned with the preservation of antisheep hemolysin the following statement appears:

"The preparation of antisheep hemolysin of high titer is such a relatively simple and inexpensive procedure that, if its preservation over long periods of time can be assured, there is little reason for the use of relatively low titer commercial preparations."

This statement was based upon practical experience in the preparation of high titer (1:15,000 to 1:40,000), antisheep hemolysin extending over a long period, during which time success has been practically uniform; but, since its publication, so many inquiries have been received as to the details of the method used that, apparently, the degree of success attained has not been the usual experience of others. In response to these inquiries, therefore, the present note detailing the method used is herewith presented.

In so far as I know, the method embodies nothing original. It is based upon that published by Kolmer,[2] although, upon consulting this reference, I find that there are minor points of difference which, however, do not appear to be of marked significance. As the method has given complete satisfaction, it is reported in full detail.

The Animal.—Healthy, adult rabbits of good size; males or females may be used but pregnant females are avoided. No special breed is necessary, nor any special diet. The animals are simply kept under ordinary conditions in clean cages.

The Antigen.—Sheep cells are used for injection on the day on which they are collected. Where a sheep is not maintained in the laboratory, abattoir cells may be used. Abattoir blood should be collected directly from the animal as it is killed, in a sterile bottle containing sufficient 5 per cent sodium citrate solution made up in normal saline to prevent coagulation. The sodium-citrate solution may be kept on hand but should be sterile and kept in a sterile bottle.

The cells are washed in normal saline in the centrifuge for six washings at high speed, being well packed each time. After the sixth washing the supernatant fluid should be tested for albumin by the heat and acid test to avoid the possibility of anaphylactic reactions in the rabbit. Should albumin be found, which has never occurred after the sixth washing, the washing should be continued until the fluid is albumin free.

Under sterile precautions, using a sterile pipette and sterile test tubes,

to one centimeter of the packed cells is added nine cubic centimeters of sterile normal (0.85 per cent) saline solution, thus making a 10 per cent suspension.

The Injection.—The rabbit is removed from the cage and its body wrapped in a towel, leaving the head and ears free. An animal box may be used if desired. The hair is removed from the ear by means of a depilatory paste (one part of barium sulphide and three parts of starch), or a commercial depilatory may be used. After the paste has been thoroughly washed off with water, the ear is washed with alcohol, which is then removed with several spongings with water. The depilatory paste renders the marginal vein easily seen and quite prominent, by reason of congestion, so that the injection is facilitated. For future injections the ear is simply washed with alcohol and water. If the vein is very small it may be made swollen and prominent by tapping with the finger or sponging with xylol. If xylol is used it must be thoroughly removed with alcohol to avoid an inflammatory reaction.

The syringe, of the Luer type, is sterilized by dry heat together with the needle, which should not be of too small a caliber. Ten cubic centimeters of the 10 per cent cell suspension are injected at a time.

After the cells have been injected, a cotton compress is held over the site of the injection for a minute or two and the animal returned to the cage.

The injections should begin as far forward in the ear as is possible in order that a new portion of the vein toward the base of the ear may be used for each subsequent injection.

Number of Injections.—Cells are injected—ten cubic centimeters each time—twice weekly (every three days). The injections should be made regularly on the appointed day. One week after the last injection, the rabbit is bled from the ear, the serum separated, inactivated for thirty minutes at 56° C. and titered.

If the titer is satisfactory, the animal is stunned by a blow at the base of the skull and bled into 50 c.c. sterile centrifuge tubes. Any method of bleeding may be used which is preferred. If the titer is not satisfactory one or two more injections may be given and the serum again tested one week after the last injection.

After its collection, the blood is allowed to stand overnight in the ice chest, the clot first being ringed, and removed by centrifugation.

Titration.—1. Stick an ear vein and collect one or two centimeters of blood in a small sterile tube. Allow to clot; ring the clot, and remove the serum by centrifugation.

Inactivate for thirty minutes at 56° C. In a large test tube place 0.1 c.c. of the serum and add 9.9 c.c. of normal saline, thus making a 1:100 dilution. Place one centimeter of this dilution in a large tube and add nine centimeters of normal saline, thus making a 1:1000 dilution, which is used for the titration.

2. Place twelve serologic test tubes in a rack and mark each with the amount of amboceptor dilution to be added as noted below.

To each tube add the amount of 1:1000 amboceptor dilution indicated: 0.05 c.c., 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.25 c.c., 0.3 c.c., 0.35 c.c., 0.4 c.c., 0.45 c.c. and 0.5 c.c.

3. To the eleventh tube, which is the hemolytic control, add 2 c.c. of normal saline.

4. To the twelfth tube, which is the corpuscle control, add 1 c.c. of normal saline.

5. To each of the first ten tubes add 0.3 c.c. of a 1:30 dilution of complement and 0.5 c.c. of 2 per cent sheep cell suspension (one part of cells to forty-nine parts of normal saline). These amounts of cells and complement are used so that the titer of the amboceptor may be determined with reference to Kolmer's quantitative method of complement fixation.

To the hemolytic control add 0.3 c.c. of 1:30 complement dilution, 0.5 c.c. of 2 per cent cell suspension and two units of an amboceptor whose titer is known.

To the corpuscle control tube add 0.5 c.c. of sheep cell suspension.

6. Incubate in the water-bath at 38° C. for one hour. At the end of this time make a reading. The unit is contained in that tube which contains the smallest amount of amboceptor just giving complete hemolysis.

If hemolysis is complete in the tube containing 0.1 c.c. of amboceptor serum and not in the tube containing 0.05 c.c., the titer is 1:10,000 (0.1 c.c. of 1:1,000 dilution being equivalent to the same amount of amboceptor serum as 1 c.c. of 1:10,000). If hemolysis is complete in 0.05 c.c. the titer is at least 1:20,000. Under such circumstances, if it is desired to ascertain the exact titer, a new set up must be made with the following amounts of amboceptor serum: 0.01 c.c., 0.02 c.c., 0.03 c.c., 0.04 c.c., and 0.05 c.c.

The titer of the serum will, of course, be influenced by the amount and dilution of the complement dose. Prior to the publication of Kolmer's test, I used 0.5 c.c. of 1:20 complement.

Preservation of the Serum.—After its collection, the serum is inactivated in bulk for thirty minutes at 56° C. and re-titered as the titer is modified by the destruction of thermolabile amboceptors. It is then preserved by the addition of an equal volume of chemically pure glycerin. Two centimeters of the glycerinated serum, representing one centimeter of the serum alone, are placed in ampules which are then sealed for storage.

The above technic is simple and embodies nothing new as far as I am aware. I have had uniform success with it in the preparation of hemolysins of not less than 1:10,000 titer. As a rule, after four injections, the titer will approximate 1:20,000. On one occasion, after four injections the titer was 1:40,000 and on another occasion, after six injections the titer was 1:60,000.

It is well known, of course, that different animals will vary somewhat in their ability to produce hemolysin but success with the method has been so uniform that I seldom inject more than one rabbit at a time unless a large amount of hemolysin is desired.

On one occasion, I found a rabbit which, in spite of repeated injections, failed to give a satisfactory serum and I once encountered a sheep whose cells were very resistant to hemolysis. It is conceivable that such cells would not prove a good antigen; this, however, is an unusual occurrence and would be compensated for in subsequent injections unless the same sheep were used

throughout which would hardly be possible as the resisting quality of the cells would soon be noted in the course of the day's work and the animal discarded.

The results as stated which have been uniform and constant with the method outlined, have, as originally noted, made it unnecessary to rely upon relatively low titer commercial preparations of antisheep hemolysin.

REFERENCES

[1] Kilduffe, R. A.: A Note upon the Stability of Preserved (Glycerinated) Antisheep Hemolysin, JOUR. LAB. AND CLIN. MED., June, 1924, ix, 9.

[2] Kolmer, J. A.: Infection, Immunity, and Specific Therapy, Ed. 2, Saunders and Co., Philadelphia, 1923, p. 80 (Method I).

The Journal of Laboratory and Clinical Medicine

EDITORIALS

The Hospital Laboratory and the Resident

A VERY definite proportion of residents look upon the laboratory service as the least interesting of their hospital experiences, approach it with reluctance, and view its termination with feelings akin to enthusiasm and relief.

There is some tendency to attribute this to the natural preference of the average resident for the more dramatic and spectacular features of certain of the clinical services, but it is possible that the responsibility for this attitude, in some measure, may rest upon the director of the laboratory and his attitude toward the laboratory work and the laboratory resident with a consequent influence upon the character imparted to the resident laboratory service.

There are few, indeed, who are able to determine their natural aptitude and general fitness for specialization in the days of their hospital term, but

even if this were always possible, it may be taken as an axiom that no specialty is so distinct as to enable it to withdraw from contact with the practice of medicine as a whole, none which can neglect the keystone of intelligent treatment—diagnosis.

If the resident comes to look upon his laboratory service as something apart and distinct from all the other departments of the hospital; if it comprises simply so many blood counts, so many smears, or so many examinations of whatever kind completed and reported as entities, separate and distinct from the patient and his condition, it is easy to see that the laboratory service may soon become devoid of interest and productive of the usual effect of monotony. Under such conditions it is not surprising that the laboratory service is productive of little that the resident finds of value in his later clinical experience.

Routine examinations there must be and a certain proportion of them must fall to the lot of the resident and, of necessity, some of his time and the attention given him must be concentrated upon the acquisition of sufficient technical expertness to assure accuracy in his work.

It is, however, of value and certainly productive of increased interest in his work and conducive to his own and the benefit of his future patients, if an endeavor is made to focus attention upon the practical side of the laboratory service and upon its daily application to the scientific practice of medicine.

It may be accepted, as preliminary premises: that clinical laboratory procedures form an essential part of clinical surveys and study; that, to no small extent, these will be made for the clinician by those skilled and expert in laboratory methods; and that their ultimate value and significance will depend entirely upon their interpretation and application to the particular case.

It may also be assumed that the average medical graduate of today will be possessed, if not of sufficient skill to perform the laboratory examinations within his province, then certainly of a mental equipment which will suffice to enable him to find and absorb the information necessary.

It would seem logical, therefore, during the service of the laboratory resident to turn his attention, not so much toward methods and technic, as toward the interpretation and clinical significance of the examinations he helps to make or sees made in the laboratory.

It may be difficult to find much of interest in a series of blood counts, but interest is not only possible but, with the right mental attitude, inevitable if each count is appreciated and regarded as an examination into a particular phase of an individual patient; if it is appreciated that upon the blood count and its interpretation the diagnosis of operative interference may, at times, depend; that it may be utilized to detect or forewarn of impending complications or influence the prognosis.

The laboratory resident should be taught to realize and to appreciate that his laboratory service is intimately and definitely associated with the clinical side of medicine. He should be encouraged to assimilate from the chart or from those in clinical contact with the case the results of the survey of the patient already made through other means, and to correlate with these

the results of the work done in his own department; to determine by study, ratiocination, or inquiry, how these results may be applied to the clinical problem and, if they are not, apparently, capable of coordination with other findings, the probable reason and significance of the disagreement.

By this means and by such methods only will the student come eventually to estimate the true value of laboratory examinations as applied to the particular case; to assort his future requisitions intelligently for his own use and the benefit of his patients; to interpret them wisely, and to realize that laboratory examinations are not and cannot be pathognomonic; that they can be "standardized" only as to technic; and that the results depend, not upon the disease, but upon the reaction of the patient to the disease, a reaction influenced by all factors which may affect the individual capacity to respond to varied stimuli.

The director of laboratories should look upon this as a most important and essential part of his duties and this conception should be the mainspring of his relation to the laboratory resident. He should be ever ready to encourage such inquiries and to assist in their elucidation; and the whole purpose and tenor of the laboratory service should be to focus attention and maintain the interest of the future clinician—not upon tests and their technic, but upon their clinical significance and interpretation.

There are few residents, indeed, who will be unresponsive; few who will not feel that the laboratory service is of concrete clinical value, and none—it is to be hoped—who will fail• to benefit in some measure and to apply the benefit to their future patients.

Occasionally, among them will be found the germ of a future clinical pathologist to carry on through others yet to come the enthusiasm impaired to himself.   —R. A. K.

BOOK REVIEWS

Chronic Intestinal Stasis*

AMERICAN writers have at no time been as thoroughly convinced of the etiologic relationship of intestinal stasis to a large proportion of those ills to which flesh is heir, as have many English writers who have become fired with the enthusiasm of those pioneers in stasis, Sir Arbuthnot Lane and Sir Arthur Keith. Some wag has said that this is because Americans have more room in which to exercise and thus prevent stasis than have our less fortunate cousins on the "straight little isle."

Intestinal stasis and intestinal toxemia undoubtedly do exist but we have come to intentionally avoid both terminologies in our diagnostic discussions. Intestinal toxemia is so easy an explanation of manifold ills and so thoroughly satisfies the patient that the use of this concept undoubtedly tends to dull one's diagnostic fervor and to promote its use when more thorough examination would have shown an entirely different cause for the complaint under study.

And thus we have perhaps gone rather to the other extreme in blocking out any consideration of stasis as an important factor in the causation of various diseases.

Jordan has attacked the problem from a radiodiagnostic viewpoint and has accumulated a wealth of interesting material. His work is abundantly and clearly illustrated, chiefly with x-ray pictures of the various conditions discussed.

While we feel that he has ascribed too many and too diverse ills primarily to intestinal stasis, we must confess that the proposition is put in a thoroughly concise and interesting way and that many of the general concepts are deserving of interest. In this country surely we have not as yet agreed that duodenal and gastric ulcer, carcinoma of the stomach, cardiospasm and carcinoma of the lower end of the esophagus are dependent ultimately upon chronic intestinal stasis nor does our understanding of carcinoma of the breast or of the upper esophagus as yet include an antecedent devitalization of the tissues through intestinal stasis.

However, as we have stated, we have perhaps gone too far in our efforts to avoid the pitfall of easy diagnosis and might well review the hypothesis championed by Jordan, provided we do not allow ourselves to be swayed too strongly thereby.

The Pathology and Treatment of Diabetes Mellitus*

TO those interested in diabetes the author requires no introduction. This
book incorporated the Goulstonian lectures on glycemia and glycosuria
and is written in the easy rhetoric of the lecture room rather than in accord-
ance with the more formal textbook technic. The first third of the book is
devoted to the physiology and pathology of the metabolism of sugar within
the body, and no matter what the future developments in treatment conse-
quent to the introduction of insulin may be, this portion of the book will pos-
sess an unaltered value.

The author has in the past applied undernutrition therapy, using his
own dietary routine, and he emphasizes that with insulin, undernutrition must
still be practiced, at least enough so that the fasting blood sugar shall reach
normal limits each day. This is the only way in which we may hope for per-
manent improvement in the functional capacity of the pancreas. The author's
treatment of coma is essentially the same as that applied in America. He has
not stressed the importance of obesity as a predisposing factor in the causa-
tion of diabetes to such an extent as does Joslin.

An appendix contains sample dietaries, tables of food values, metabolism
charts, description of laboratory methods, and a consideration of the keto-
genic-antiketogenic ratio.

The volume, particularly that portion dealing with the physiology of car-
bohydrate metabolism, should be valuable to all who have any special interest
in the study or treatment of diabetes.

Diabetes, Its Treatment by Insulin and Diet. A Handbook for the Patient†

WHEN we consider the relatively large number of brochures on diabetes
for the use of physician and patient alike, published within the last few
years, especially since the advent of insulin, we cannot but remark on the
individualization which is apparent in the works by the various authors. Of
those which the reviewer has seen so far, all are sufficiently different to be of
individual interest, and one who is particularly interested in the treatment of
diabetes will do well to have an assortment of these small books at his disposal.
Each author presents his own method of attacking the problem.

In Petty's handbook we may mention the following outstanding features
which we find discussed perhaps better here than in books by other authors.

The method of estimating the optimal diabetic diet is described with un-
usual clarity and is considerably simplified. While of interest to the patient,
this should be read particularly by the physician.

In addition to the short chapter on insulin we find a description of the
technic of administering insulin at home, care of the syringe and needle,

measurement in the syringe, etc. The simple menus and the daily diet schedule follow quite closely those originally drawn by Joslin.

We are glad to note here for the first time that the so-called 5 and 10 per cent vegetables are denominated 3 per cent and 6 per cent, which is their actual percentage when prepared for the table. Five and 10 per cent fruits are, of course, still designated as such.

There are several unusual and useful food tables, such as one showing foods of high fuel value, medium fuel value and low fuel value; one on the salt content of foods, and one showing excess of acid-forming or base-forming elements in foods.

Hemorrhoids*

TWO major motives have impelled the author to write a small monograph on hemorrhoids; first, the recognized confusion not only in the mind of the laity but also in a large proportion of physicians as to the true significance of the term "piles" (various diseases of the rectum and anus being grouped under this single heading); and second, as a vehicle for the description of his technic in the injection treatment of internal hemorrhoids. His treatment is distinctly conservative in that it avoids the necessity for extensive operation and usually saves the patient the expense and the time wasted in the hospital. The sections on surgical anatomy and physical diagnosis are well illustrated and a study thereof will serve to clear up misconceptions among those practitioners who are not strictly proctologists.

The advantages of the treatment of hemorrhoids by injection are not only the considerable saving of time and expense to the patient, but also the avoidance of general anesthesia, scarcely even a local anesthetic being required; the fact that symptoms cease immediately; that it is a safe procedure in pregnant women or individuals with advanced cardiac or renal disease; that there is as a rule no after-pain; that complications are less common; and that by this method hospital beds are left free for more urgent cases.

Pathological Technique†

THE histopathologists and bacteriologists *vade mecum* of laboratory methods, to which are added chapters on examination of the blood, urologic technique, examination of the cerebrospinal fluid, of sputum and a description of the technic of postmortem examination. This is a volume which, being brought up-to-date with each new edition, has survived for twenty-seven years the date of its first publication. It needs no introduction to the profession.

*Hemorrhoids. By Arthur S. Morley, F R C S, Eng Cloth. Pp 114 Henry Frowde and Holder and Stoughton, London, 1923

†Pathological Technique A Practical Manual for Workers in Pathological Histology and Bacteriology. By Frank B Mallory, M D, Pathologist to the Boston City Hospital and James B. Wright, M.D., Pathologist to the Mass General Hospital. Eighth edition, revised and enlarged. Octavo of 666 pp., with 180 illustrations Cloth Price $6.50 W B Saunders Co, 1924.

The American Society of Clinical Pathologists

Dr. John A. Kolmer, Philadelphia, Pa.
President

Dr. Frederick E. Sondern, New York
1st Vice President

Dr. W. F. Thomson, Beaumont, Texas
2nd Vice President

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The Next Annual Meeting Will Be Held in Philadelphia May 20-23, 1925
Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 20, 21, 22 and 23, 1925, at the Benjamin Franklin Hotel, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering this year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for at: tending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.

Members of the A. S. C. P. are requested to notify the secretary of any changes in their addresses.

·· Clinical pathologists desiring to apply for membership may communicate with· the secretary, Dr. Ward Burdick, Children's Hospital, Denver, Colorado.·· ·

STANDARDS FOR CLINICAL PATHOLOGIST

One of the questions to be taken up at the business meeting at the next annual convention of the American Society of Clinical Pathologists is that relating to the qualification of clinical pathologists to practice this specialty. Considerable attention has been given by the society to this subject. In order to gather the consensus of opinion, the following letter and enclosures have been sent to every member.

Dear Doctor:

The incompetent and irresponsible laboratory is one of the evils now besetting the Medical Profession. The American Society of Clinical Pathologists has assumed the task of eradicating this excrescence on our professional body by endeavoring to institute standards of qualifications for those assuming to make laboratory diagnosis.

A preliminary report on laboratory standardization was submitted at the Rochester meeting by Dr. F. E. Sondern, Chairman of the Committee on Standardization (published in the January, 1925 number of the Journal of Laboratory and Clinical Medicine). Since then the committee has spent much time and labor in evolving a tentative plan for consideration at our next meeting. In order that every member may have an opportunity to study the details of the proposed plan, a copy is herewith enclosed. Appended thereto is a questionnaire which I would urge you to use for a free expression of your views and counsel, and as a guidance for the formation of a more permanent scheme. Let me hear from you at your early convenience.

I might add that the Philadelphia meeting promises to be a pronounced success. From present indications we shall have a wealth of interesting papers. If you have not already sent in the title for a paper you desire to present, do it now. One day of the convention will be devoted entirely to questions affecting the status of our members and our specialty.

Hoping to hear from you soon, I am

Fraternally yours,
(Signed) Ward Burdick,
Sec'y.-Treas.

A SYSTEM OF LABORATORY APPROVAL PROPOSED TO THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

By the Committee on Standardization

At the last meeting of the Society it was proposed that your Committee on Standardization frame the conditions under which the Society may issue Certificates of Approval to the members requesting the same.

The arguments in favor of the establishment of a method of approval of laboratories by a representative body such as the American Society of Clinical Pathologists are detailed in the report of your Committee adopted at the last meeting in Rochester, Minnesota, in June, 1924, which was essentially as follows:

It is an established fact, that a large number of clinical laboratories are conducted by persons absolutely unqualified by training and experience.

This state of affairs has, during recent years, engaged increasing attention particularly of those interested in public health, and a remedy is eagerly sought.

Practically speaking, clinical laboratory work is the result of a demand of the physician for aid in diagnosis, and were physicians as a class exacting in the laboratory service rendered, the existing evil would soon come to an end.

These constantly increasing justified complaints relative to inefficient and misleading laboratory service particularly in our larger cities must have the serious attention of such

bodies as our Association, and a remedy should be found to make every probable legislative control unnecessary or to guide such control if in public opinion it should become necessary.

Specific happenings in this connection may tend to emphasize the brief statements which have been made. Some eight years ago or more the New York City Commissioner of Health, Haven Emerson, called attention to the large number of complaints of incompetent work done by many private laboratories in New York City and expressed the hope that the New York Academy of Medicine might do something to remedy this condition. In consequence, the Public Health Committee of the Academy made an investigation and as a result of this, advised that the Board of Health frame rules and regulations under which private laboratories should be allowed to operate. Beyond the requirement of a license, nothing has been done to date.

About the same time a similar complaint was made by officers of the American Medical Association to some of the private clinical pathologists of Chicago, with an unofficial request that the laboratory men themselves establish an organization for laboratory standardization and control, which, if suitable and efficient, should win the support of the American Medical Association. A determined effort in this direction was made under the leadership of Hektoen and others, but the World War prevented the success of this undertaking. It was also about this time that a study of this problem was made by the New York Bureau of Municipal Research, and in an exhaustive report published by this body the authority of the City Department of Health in the matter was demonstrated under existing law and compulsory control was strongly advocated in the interest of public health. The hospital surveys under the auspices of the American College of Surgeons, and the activities of the Council of Medical Education and Hospitals of the American Medical Association, have given increasing attention to laboratory personnel and equipment.

Enough has been said to prove that the danger of existing laboratory conditions is generally recognized and therefore, it scarcely seems necessary to review in detail evidence of its actual existence. There is no one active in clinical pathology who cannot detail a number of laboratories conducted by insufficiently trained technicians, and even by medical graduates who have scant knowledge of laboratory technic and employ incompetent technicians to do the actual laboratory work. It is also not uncommon to find hospital laboratories equally inefficient for like reasons, to say nothing of instances when a competent clinical pathologist, through stress of private work, exerts no control over his inefficient institution laboratory.

Admitting existing conditions, what can our Society do to remedy these evils, and what will happen if the issue is evaded? These are important questions with professional and economic significance which confront the qualified physician who specializes in clinical pathology, and it would seem urgent for our Society to assume as one of its legitimate functions, the duty of at least attempting a solution. While self-appointed censorship is on the whole an undesirable method, it does not stand without precedent, and if no other scheme is available, it may be necessary to come to it.

It is suggested that a method shall be devised by which the laboratories of members of the Society may, on request, secure what may be known as a "Certificate of Approval" of the American Society of Clinical Pathologists. This method should include qualifications of personnel, standardization of laboratory procedure controlled by inspection, periodic report on submitted specimens or by such other method as the Society may deem proper. The details of these requirements and standards might be relatively simple at first, to be improved and perfected as the plan develops and its need and desirability become more evident. At all events, this official approval should be of such high standard as to secure at sight the unqualified endorsement of such bodies as the American Medical Association, the American College of Surgeons, etc.

This procedure, to be sure, will not eliminate the inefficient laboratory because it will not succeed in making the careless or incompetent physician or the lay public pay proper attention to the securing of efficient laboratory service. It will however, convince the thinking medical men of the country and the leading organizations of which they are members, that a laboratory standard has been established which they cannot ignore.

We live in an era of profuse legislative activity in the control of almost every human endeavor, and if this should extend to a consideration of laboratories, aside from such activity as a purely medical professional function, as it well may, the existence of an established standard endorsed by leading medical organizations might easily shape a proper course of action. In the absence of such organization, however, the legislation might easily grow to bizarre proportions, of which we have so many examples in other fields at the present time.

The only existing practical standardization and control of public health laboratories is that of the New York State Department of Health. This plan was conceived and elaborated by Dr. A. B. Wadsworth, Director of the Laboratories of that Department and carried out by and under the supervision of Dr. Ruth Gilbert, head of the Diagnostic Laboratory of the same. The system is of decided value to the Department in estimation of the reliability of the laboratories throughout the State and their value in safeguarding public health. It is in the main characterized by courteous cooperation and constructive criticism in which way it renders a valuable service to the laboratory itself; and its existence without any doubt urges higher laboratory standards.

A similar system of voluntary submission to laboratory control exerted by this Society may easily be the means of convincing the thinking members of the medical profession that an absolute laboratory standard has been established.

It will be of little or no use unless a large proportion of the efficient laboratories of the country join in the movement and they will not do so unless the control is exercised by competent authority in a tactful, courteous and constructive manner. In addition it must also be recognized by the large influential medical societies, and their emphatic publicity is necessary to make those disinclined submit to approval in the interest of the common good.

Your committee recommends the following:

CONDITIONS UNDER WHICH THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS WILL ISSUE CERTIFICATES OF APPROVAL TO THE LABORATORIES OF MEMBERS REQUESTING THE SAME.

Certificates shall be issued for the term of one year, unless revoked, for those branches of clinical pathology for which the applicant has applied and in which the laboratory is considered proficient. Certificates to be in form as per Exhibit I.

Proficiency is to be determined by the Society as its appointed representatives elect. Full cooperation is expected of the applicant, and courteous, just and constructive criticism is to be the rule of the Society.

As a basis for approval, the applicant is to submit the details requested in the application as per Exhibit II. To furnish quarterly reports on the work done by the laboratory during the three months just passed, on suitable blanks as per Exhibit III. To subscribe to certain rules and standard methods and to furnish details of technic employed as per Exhibits IV.* (It will be noted that for the present the latter apply only to examinations which have to do with communicable disease and the examinations of milk and water as these are considered most important. It is expected that similar details and agreements will be arranged later to cover all the branches of clinical laboratory work.

The laboratory shall be subject to inspection by competent authority to verify the various agreements to which the Director has subscribed.

From time to time test specimens will be sent to the laboratory for examination and report which are to have the same attention in examination and report as specimens submitted to the laboratory in the regular course of its work.

Applicants for approval are also expected to subscribe to the following rules:

The Director of the clinical laboratory must be a licensed physician and all diagnostic opinions of the laboratory must be framed by him or with his ap-

*Exhibit form IV eliminated from this preliminary survey.

proval. In his absence this shall be done by an equally qualified associate or substitute. The Director shall have complied with all federal, state, county and city regulations which apply to laboratories. It. shall be considered unethical to advertise the laboratory in any way other than that having the approval of the Society. Directors, consultants and the like shall not be announced unless they are actually on duty in the laboratory. The division of fees with physicians and others who secure work for the laboratory is prohibited. The equipment of the laboratory shall be such that the proper technical procedures can be carried out in every instance and includes ready access to the necessary scientific books and periodicals.

A fee will be expected from each laboratory requesting approval to cover the acutal expense to the Society, this to be as reasonable as efficient work allows.

The success of this plan depends on the complete support of the influential laboratory men of the country. With such cooperation the Society can appeal to the American Medical Association, the American College of Surgeons and similar bodies for several purposes, viz.:

To endorse this effort to secure known reliable laboratory service for physician and patient; to publish lists of the approved laboratories, and chiefly to educate the physician to the need of approved laboratories, and to point out the danger of accepting the work of individuals not approved.

In event of proposed legislation for the control of laboratories if necessary at all, this plan, seasoned by then, can be offered as a compulsory instead of a voluntary method.

It is not hoped that this method of approval and control will eliminate the unfit worker or laboratory, but if the plan is given sufficient publicity those not approved will have difficulty in defending their position.

COMMITTEE ON STANDARDIZATION

FREDERICK E. SONDERN, M.D., Chairman, *New York City, New York.* ·
A. V. ST. GEORGE, M.D., *New York City, New York.*
W. F. THOMSON, M.D., *Beaumont, Texas.*
GEORGE IVES, M.D., *St. Louis, Missouri.*
THADDEUS WALKER, M.D., *Boston, Massachusetts.*
RUTH GILBERT, M.D., *Albany, New York.*
HARRY J. CORPER, M.D., *Denver, Colorado.*

QUESTIONNAIRE

1. Do you approve of the plan outlined by the Committee on Laboratory Standardization?
2. If not, state objections.
3. What modifications or additions would you suggest?
4. Remarks:
5. Further Comments—(write freely).

NAME ....

ADDRESS ....

....

Some very interesting replies have been received in response to the questionnaire which will be discussed in a future issue.

APPLICATION FOR CERTIFICATE OF APPROVAL

Date ....

To the Committee on Standardization of the American Society of Clinical Pathologists:

I, ... , M.D.,

located at ....
  P. O. Address   City   State

hereby apply for a certificate of approval of my qualifications in Clinical Pathology on the grounds of the following education and experience:

SCIENTIFIC AND MEDICAL EDUCATION

NAME OF SCHOOL	LOCATION	TIME		DEGREE
		FROM	TO	

SPECIAL COURSES

INSTITUTION	LOCATION	TIME		
		FROM	TO	

PRACTICAL EXPERIENCE

LABORATORY	CHARACTER OF WORK DONE	TIME	
		FROM	TO

The laboratory staff consists of ... ...

....

....,... .. ..

....

. .

....

....

(EXHIBIT II.)
(Obverse side)   . (OVER)

The laboratory has the following equipment in good condition:

....

....

....

....

....

....

....

The scope of work of the laboratory includes ....

....

....

....

....

Indicate by means of a cross (x) the classes of service you are performing.

Private... Hospital... Medical School... Municipal ... County... State... Federal...

I hereby agree that:

1. The work will be done strictly in accordance with the code of ethics of the American Society of Clinical Pathologists.

2. The technic employed will be that approved by the Society.

3. An accurate record will be kept of the daily work.

4. All stained tissue sections will be kept on file.

5. The laboratory will be open for inspection to representatives of the Society.

6. Specimens sent from the Society for testing the efficiency of approved laboratories will be examined and reported.

....
Signature of Applicant.

CERTIFICATE OF APPROVAL

Issued by

The American Society of Clinical Pathologists

_____

This is to certify, That

_____ **M. D.**

has demonstrated to the Committee on Standardization of The American Society of
Clinical Pathologists that he is competent to perform the necessary examinations and
interpret the findings in the following branches of Clinical Pathology:

Clinical Microscopy,

Clinical Chemistry,

Bacteriology,

Serology,

Tissue Diagnosis.

COMMITTEE ON LABORATORY
STANDARDIZATION

_____

[SEAL]   _____

_____

Dated _____   _____

_____

_____
President.

_____
Secretary.

(EXHIBIT I)

A Four Day Convention

Owing to the large number of excellent scientific papers that will be presented at the next convention and in order to allow more time for the discussion of business matters, the Executive Committee of the American Society of Clinical Pathologists has extended the convention one day ahead, beginning Wednesday, May 20th, and lasting through Saturday, May 23, 1925. All reservations at the Benjamin Franklin Hotel have been automatically changed to include the new date.

Program of the Fourth Annual Meeting of the American Society of Clinical Pathologists

Benjamin Franklin Hotel, 9th and Chestnut Streets, Philadelphia, Pennsylvania.

WEDNESDAY, MAY 20, 9 A. M. (PROMPTLY)

Call to Order—Short Business Session

Scientific Program

The Preparation of Solutions of Dextrose for Intravenous Administration, by Dr. Ralph G. Stillman, New York City, N. Y.

Utilization of Carbohydrates, by Dr. W. G. Karr, Philadelphia, Pennsylvania (by invitation).

Diagnostic Value of Spinal Fluid Sugar Content, by Dr. W. Parker Stowe, Rochester, New York.

Alveolar CO_2 Tension and Acetone in the Expired Air in Acidosis, by Dr. Paul Roth, Battle Creek, Michigan.

WEDNESDAY, MAY 20, 2 P. M. (PROMPTLY)

Aids to Urinalysis in a Large Hospital, by Dr. C. Pons and Dr. E. B. Krumbhaar, Philadelphia, Pennsylvania (by invitation).

A New System of Duplicating and Placing Laboratory Reports in the History, by Dr. Frank W. Hartman, Detroit, Michigan.

Value of Method of Keeping Records of Tissue Examinations, by Dr. L. A. Turley, Norman, Oklahoma.

A New Type of Instrument for the Estimation of Hemoglobin, by Dr. C. E. Roderick, Battle Creek, Michigan.

Some Useful Apparatus, by Dr. Max Shaweker, Dover, Ohio.

The Organization of a Metabolism Laboratory, by Dr. William H. Stoner, Philadelphia, Pennsylvania.

Endothelioma; the Use and Misuse of the Term, by Dr. James B. Bullitt, Chapel Hill, North Carolina.

Myocardial and Endocardial Changes in Cardiac Defects, by Dr. Maude E. Abbott, Philadelphia, Pennsylvania.

THURSDAY, MAY 21, 9 A. M. (PROMPTLY)

Autopsy Report of Two Cases of Thymic Death During Surgical Operations, by Dr. Jessie W. Fisher, Middletown, Connecticut.

Some Enzyme Studies with Desiccated Gonadal Tissue, by Dr. Herman Sharlit and Dr. William G. Lyle, New York City, N. Y.

A Comparison of Enzyme Actions of Tumors and Normal Tissues, by Dr. K. George Falk and Helen Miller Noyes, New York City, N. Y., (by invitation).

A Double Hydrogen Electrode System for the Determination of Hydrogen-Ion Concentration, by Dr. George H. Meeker and Mr. Bernard Oser, Philadelphia, Pennsylvania, (by invitation).

Observations on the Dick Test, Toxin Immunization and Serum Treatment of Scarlet Fever, by Dr. John A. Murphy, Philadelphia, Pennsylvania. (by invitation).

Pneumococcus Antibody Solution, by Dr. Frank M. Huntoon, Glenolden, Pennsylvania.

Variation in Size of Red Blood Corpuscles and High Color Index with little or no Anemia, (Lantern Slides), by Dr. Mortimer Warren, Portland, Maine.

THURSDAY, MAY 21, 2 P. M. (PROMPTLY)

Normal and Pathologic Basal Metabolic Rate in Obesity, by Dr. Harry M. Jones, Chicago, Illinois.

Studies on Blood Cultures with Special Reference to the "Massive" Method, by Dr. Herbert Fox and Dr. William G. Leaman, Philadelphia, Pennsylvania.

A Comparative Study of Liver Functional Tests, by Dr. A. I. Rubenstone and Dr. Louis Tuft, Philadelphia, Pennsylvania.

The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method, by Dr. B. S. Parks, Philadelphia, Pennsylvania, (by invitation).

Studies on the Bacteriology of the Urine in Cooperation with Catheterization of the Ureters, by Dr. Robert A. Keilty, Danville, Pennsylvania.

Study of Cases of Acute Leukemia and Acute Mononucleosis, by Dr. B. L. Crawford, and Dr. Harold W. Jones, Philadelphia, Pennsylvania.

Blood Counts in Mississippi, by Dr. Leon S. Lippencott, Vicksburg, Mississippi.

THURSDAY, MAY 21, 7 P. M. (PROMPTLY).

Reaction After Typhoid Vaccination, by Dr. Henry J. Nichols, Washington, D. C.

The Measurement of Cloudiness in Liquids, by Dr. William G. Exton, Newark, New Jersey.

Studies in Embalming Fluids in Relation to Gross and Histological Tissue Examinations, by Dr. John A. Kolmer and Dr. Fred Boerner, Philadelphia, Pennsylvania.

The Specific Inflammatory Reaction of Immunized Animals (Arthus Phenomenon), by Dr. Eugene L. Opie, Philadelphia, Pennsylvania, (by invitation).

Methods of Staining Tubercle Bacilli, by Dr. H. J. Corper, Denver, Colorado.

FRIDAY, MAY 22, AT 9 A. M. (PROMPTLY)

Value of the Leucocyte Fragility Test in the Prognosis of Pneumonia, by Dr. C. Pons and Dr. E. P. Ward, Philadelphia, Pennsylvania, (by invitation).

Sedimentation Rate of Erythrocytes, by Dr. H. N. Cooper, Watertown, New York.

Standardization of Tuberculin, by Dr. Joseph D. Aronson, Philadelphia, Pennsylvania, (by invitation).

Bronchial Spirochetosis, by Dr. Thomas L. Ramsey, Toledo, Ohio.

Etiological Studies in Psoriasis, by Miss Mary Marcus, Philadelphia, Pennsylvania, (by invitation).

Squamous Cell Carcinoma of the Gall Bladder, by Dr. Frank W Hartman, Dr. W E. Johnson, Detroit, Michigan.

The Technic of the Practical Application of the Pathogen Selective Cultural Method, by Dr. A. I. Rubenstone, Philadelphia, Pennsylvania.

FRIDAY, MAY 22, 2 P. M. (PROMPTLY).

A System of Laboratory Approval Proposed to the American Society of Clinical Pathologists, by Dr. Frederick E. Sondern for the Committee on Standardization

Discussion to be opened by Dr. Ruth Gilbert, Dr. C. Y. White (by invitation) and Dr. S. R. Haythorn.

Open Discussion

How Can We Best Promote the Objects Contained in Article II of Our Constitution? By Dr. Herman Spitz, Nashville, Tennessee.

Suggested Method to be Followed in Developing a Standardized Course for Medical Technicians, by Dr. Walter E. King, Detroit, Michigan

FRIDAY, MAY 22, 7 P. M. (PROMPTLY)

Annual Dinner in Ball Room of the Benjamin Franklin Hotel.

Presidential Address

The Functions of a Hospital, by Dr. George H. Meeker, Dean of the Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

SATURDAY, MAY 23, 9 A. M. (PROMPTLY)

Business Session

SATURDAY, MAY 23, 2 P. M. (PROMPTLY)

The Clinical Significance of Anticomplementary Serum, and Spinal Fluids in the Wassermann Reaction, (Kolmer Modification), by Dr. A. H. Sanford, Rochester, Minnesota.

A Standardized Wassermann Report, by Dr. A. J. Casselman, Camden, New Jersey.

The Present Status of the Kolmer Complement-Fixation Test for Syphilis as Determined by a Comparison with Numerous Other Methods, by Dr. Robert A. Kilduffe, Atlantic City New Jersey.

Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, by Dr. Robert G. Owen, Detroit, Michigan.

Kahn's Precipitation Reaction as Compared to Kolmer's Complement-Fixation Test, by Dr. A. S. Giordano, South Bend, Indiana.

A Clinical Study of the Kolmer and Kahn Reactions in Syphilis, by Dr. R. L. Kelly, Louisville, Kentucky, (by invitation).

The Meinecke Reaction as Compared with the Wassermann in One Thousand Specimens of Blood Sera. (Lantern Slides), by Dr. A. M. P. Saunders, Dunning, Illinois.

Scientific Exhibit

Microscopic and Other Specimens Relating to Tropical Medical Problems, by Dr. J. M. Feder, Santo Tomas Hospital, Panama.

CLINICAL AND EXPERIMENTAL

MICROSCOPICALLY DEMONSTRABLE FAT IN NORMAL HUMAN HEART MUSCLE*

By A. M. Master, M.D., New York

IN June, 1922, two of us worked at New York Hospital[1] in an endeavor to correlate the gross and microscopic condition of the heart as obtained at autopsy with the electrocardiograph curves taken just before death. One of the stains which was employed was scharlach r and we found that minute red droplets appeared in practically every heart that we examined. We were unable to determine whether this indicated fatty degeneration or whether it was a normal finding. In order to decide this point, we obtained hearts from individuals who had met sudden violent death by bullet or stab wounds, etc., and who at autopsy showed no abnormality that was apparent to the unaided eye.

Thirteen hearts were examined from individuals ranging from eight to fifty-six years, the average age being thirty-two years. Sections were taken from each ventricle, septum, and auricle. The tissue was fixed in formalin and within twenty-four hours frozen sections were stained with a saturated solution of scharlach r dye in equal parts of 70 per cent alcohol and pure acetone. The technic followed was that given by Mallory and Wright.[2] At the conclusion of the experiments we stained one of the hearts with osmic acid (formalized material placed in Marchi's fluid and cut by the freezing microtome). The results obtained were similar to those with scharlach r. The table and photomicrographs describe our findings. In regard to the scharlach r we found small red droplets of varying sizes in the sarcoplasm of the cardiac muscle cells. These were arranged in longitudinal and transverse rows,

the longitudinal droplets appearing between myofibrillae. The number of granules varied. Some hearts were stained diffusely and uniformly, some scarcely at all, and commonly there were groups of cells which took the stain well, while in the immediate vicinity were cells which contained little, if any, fat.

The so-called pigment of brown atrophy, which is located at the poles of the nucleus and is supposed to be an indication of senility of the muscle, was stained yellow, yellow brown, or golden brown with scharlach r. At first I found it difficult to distinguish between it and the diffuse fat droplets, but
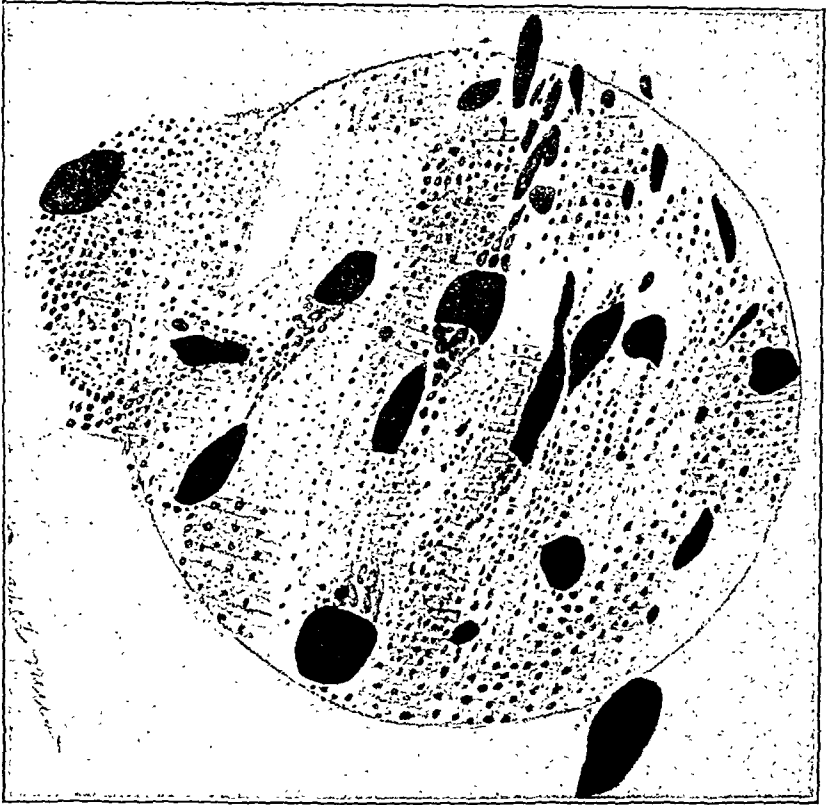
Case A.—Scharlach r stain. Carmine red fat droplets (black in half-tone) in longitudinal rows between the muscle cell fibrillae and arranged transversely (on either side of Krause's membrane). Pigment of brown atrophy at poles of nuclei.

after some experience I was able in practically every case to differentiate between the coarser brownish bipolar granules and the smaller red fat droplets distributed diffusely throughout the cell and arranged in longitudinal and transverse rows. With osmic acid the bipolar pigment was stained light brown, whereas the fat granules were intensely black.

Hofbauer,[3] in 1905, described visible fat in normal human fetal heart muscle. Bell,[4] in 1912, first showed that visible fat is normally present in the cardiac muscle of the common laboratory mammals. He also demonstrated that the quantity of visible fat is increased when fatty foods are given and

diminished when the animals are starved. Wegelin[5] found fat in the cardiac tissue of rats. He also examined the heart of an insane man who had jumped out of the window and found fat in quantities, although the organs appeared to be normal at autopsy. He expressed the belief that fat could be demonstrated microscopically in normal human heart muscle. Eyselein[6] of Berlin did not agree with this.

H. Hays Bullard,[7] in 1912, stated that although scharlach r was not specific for neutral fat (scharlach r and sudan III stain neutral fats, fatty acids, soaps and lipoids with varying degrees of intensity) and although he did not believe that all the colored droplets in mammalian cardiac muscle were neutral fat, yet he thought that most of them undoubtedly were. He showed that these bodies were completely dissolved by absolute alcohol and other fat solvents, that they stained characteristically with scharlach r, and red (not blue) with Nile blue sulphate and Nile blue chlorhydrate. The red granules
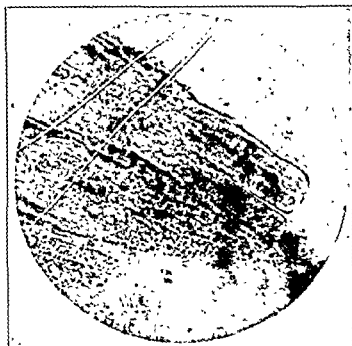
Case A.—(Ventricular septum.) Scharlach r stain. Carmine red fat droplets (black in microphotograph) in longitudinal rows between the muscle cell fibrillae and arranged transversely (on either side of Krause's membrane)

did not stain with basic analine dyes and were not rendered insoluble by potassium bichromate. These and other properties made it appear certain to Bullard that the droplets were neutral fat and not phospholipines, cholesterinesters, etc., which may occur normally or abnormally in cardiac muscle.

In a subsequent communication, Bullard[8] showed that there is microscopically demonstrable fat in the normal cardiac tissue of rats, cats, dogs, hogs, oxen and sheep. More than 200 animals were investigated. The fat droplets in the sarcoplasm were arranged in rows between the muscle fibrillae and in transverse lines in segment J on either side of the membrane of Krause. He also noted fatty fibers side by side with nonfatty areas. However, in other cases all the cells showed a uniform diffuse mottled appearance.

Bullard gave conclusive reasons for believing that visible droplets of neutral fat occur in physiologic circumstances in the cardiac muscle fibers of mammals. I quote verbatim:

MICROSCOPICALLY VISIBLE FAT IN NORMAL HUMAN HEART MUSCLE

CASE NO.	AGE	SEX	COLOR	CAUSE OF DEATH	STATE OF NUTRITION AT AUTOPSY	PRESENCE OF DIFFUSE RED FAT DROPLETS IN THE MUSCLE CELL BETWEEN THE FIBRILLAE ARRANGED IN LONGITUDINAL AND TRANSVERSE ROWS	PRESENCE OF GOLDEN BROWN PIGMENT AT THE POLES OF THE NUCLEI, I.E., PIGMENT OF BROWN ATROPHY
A	33	Male	White	Blackjacked i.e. skull fracture, died within few hours	Good	Large quantity	Large quantity
B	45	Male	White	Stab-wound of heart. Died immediately.	Good	Large quantity	Large quantity
C	45	Male	White	Fracture cervical vertebrae. Died in few hours	Good	Slight or moderate amount	
D	23	Female	White	Cerebral hemorrhage died either immediately or within few hours	Good	Present in some areas	Large, coarse, brown, bipolar pigment
E	40	Male	White	Fracture of skull. Died in 3½ hrs.	Very good	Abundance of granules	Good many granules
F	31	Female	White	Clinical diagnosis not made	Good	Abundance of granules	Good many granules
G	20	Male	White	Shot to death. Died within 1 hr.	Somewhat emaciated	Abundance of granules	Good many granules
H	42	Male	White	Acute alcoholism	Good	Very few granules	Very many granules
I	32	Female	Black	Died in few hrs. from hemorrhage after attempted criminal abortion	Good	Moderate amount	Very many granules
J	34	Female	Black	Acute alcoholism	Good	Great many granules	Very many granules
K	56	Male	White	Cerebral hemorrhage from blow	Good	Great many granules	Very many granules
L	15	Male	White	Shot to death. Died instantly	Good	Many granules	Moderate amount
M	8	Male	White	Run over by truck. Died in few hrs.	Good	Many granules	Moderate amount

·(1) "In each of several well-known species here studied, visible fat is found with great regularity in the heart muscle of all individuals, whether fetal, young or adult. Very few, if any, of the cardiac fibers are absolutely free.

(2) "The fat droplets are not found in the contractile elements. They occupy a definite position in the sacroplasm and do not interrupt the continuity of Krause's membrane.

(3) "There are found in the muscle fibers neither degenerative changes nor any other evidence of any pathological alteration of structure.

(4) "There is no evidence of functional disturbance of the cardiac muscle which contains these droplets.

(5) "The quantity of fat is variable, is decreased in inanition and increased by fatty foods."
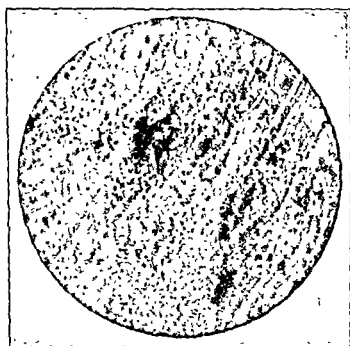
Case D.—Osmic acid stain. Results similar to scharlach r. (See Case A.)

Bullard states that the wide divergence of opinion in regard to the fat content of normal cells is due to differences of technic employed. He declares that formalin as a fixative is not always to be relied upon; in fact, fresh sections may be loaded with droplets, whereas formalin fixed tissue contains little fat.

He utilizes a 20 per cent solution of formalin[9] rendered isotonic with 0.75 gm. NaCl per 100 c.c. liquid. Tissues are fixed for one-half to five hours and then cut on the freezing microtome. If this procedure is followed, the quantity of fat does not differ from that obtained in fresh tissues. By means of Herxheimer's alkaline alcoholic solution of scharlach r, fat may often be demonstrated in larger amounts than by the simple alcoholic solution of the dye. (This stain[10] is a saturated solution of scharlach r in 70 per cent alcohol to which 2 gm. of NaOH are added to every 100 c.c. of fluid. Precipitates must be avoided.)

Bullard examined a number of human hearts which were normal in color and showed "no cloudiness, opacity, or yellowish white appearance." He found a moderate amount of fat arranged in a way similar to that demonstrated in animals. However, whether these were normal human hearts, one cannot say.

Within the last few years several articles[11] have appeared in the literature that tend to prove that the pigment of brown atrophy is an endogenous melanin and that some of the red droplets in the cell brought out by scharlach
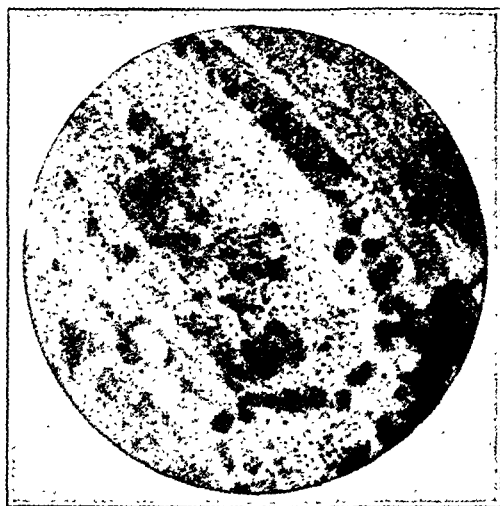
Case J.—Scharlach r stain. Fat droplets in longitudinal rows between the muscle fibers and in transverse alignment on either side of Krause's membrane.

r are of an exogenous lipochrome. But this lipochrome is stained a deep blue by Nile blue stain, not red as the fat granules are. Other writers, too, state that the pigment can be separated from the fat.

SUMMARY

In human cardiac muscle microscopically visible fat is normally present. The fat resides in the sarcoplasm between the muscle fibrils, and is arranged in longitudinal and transverse rows. Apparently, the amount has no relation to the state of nutrition at the time of death; neither has the age of the individual (eight to fifty-six years), the color, nor the sex.

The picture presented by the diffuse red fat droplets (scharlach r) or the black granules (osmic acid) closely resembled the classical illustrations that many of the textbooks[12] on pathology utilize to depict fatty degeneration of the heart. True fatty degeneration, e.g., the "tiger heart," is easily recognized. Generally the number and size of the granules differentiates the two conditions.

In all experimental work in which sections of cardiac tissue are stained for fat, normal conditions should be kept in mind and controls performed whenever possible. In the investigation of twelve pathologic hearts we found

only one organ demonstrating fatty degeneration. Another writer[13] performing work similar to ours reported "marked fatty degeneration" in every case.

Pathologists were shown our scharlach r sections of normal hearts; without knowing the history of the cases, they stated that the hearts were pathologic, i.e., that they were in the condition of fatty degeneration. They were surprised to learn that the sections were from healthy human hearts.

This investigation illustrates the necessity for experimental investigation of normal human tissues. Pathologic conditions are in many instances known far better than the ordinary healthy state.

The so-called pigment of brown atrophy of the heart, which is supposed to be an indication of degenerative processes, e.g., senility, was found in moderate amounts in two healthy boys, one eight years old, and the other fifteen.

(I wish to express my sincere thanks to Drs. Nonidez and Papinicolau of Cornell University Medical College for their complete cooperation and suggestions and to Mr. H. Wittner who drew the plate.)

REFERENCES

[1]Pardee, H. E. B., and Master, Arthur M.: Electrocardiograms and Heart Muscle Disease, Jour. Am. Med. Assn., January, 1923, lxxx, 98-101.

[2]Mallory and Wright: Histological Technique, Ed. 7, p. 107.

[3]Hofbauer, J.: Die Physiologische Fettinfiltration des Fetalen Herzens. Anat. Anz., 1905, xxvii, 426.

[4]Bell, E. T.: The Interstitial Granules in Fatty Metamorphosis of Striated Muscle, Jour. Path. and Bacteriol., 1912, xvii, 147.

[5]Wegelin, C.: Ueber alimentare Herzmuskelverfettung, Berl. klin. Wchnschr, 1913, 1, 2125 and 2190.

[6]Eyselein, K.: Untersuchung über den Fettgehalt der Herzmuskulatur, Virchow's Arch. f. path. Anat., 1914, ccxviii, 30.

[7]Bullard, H. Hays: The Microscopical Demonstration of Fats in Tissue Sections. Jour. Med. Research, 1912, xxvii, 55.

[8]Bullard, H. Hays: On the Occurrence and Physiological Significance of Fat in the Muscle Fibres of the Normal Myocardium and Atrioventricular System. Am. Jour. Anat., 1916, xix, 137.

[9]Mann, G.: Physiological Histology, Oxford, Clarendon Press, 1902, p. 88.

[10]Herxheimer, G.: Ueber Fettfarbstoffe. Deutsch. med. Wchnschr., 1901, xxvii, 607.

[11]Dolley, David H., and Guthrie, Frances V.: The Pigmentation of Heart Muscle, Jour. Med. Research, 1921, xli, 289.

[12]MacCollum, W. G. A.: Text Book of Pathology, 1918, p. 80.

[13]Willius, F. A.: Mayo Clinic Collected Papers for 1918, p. 140.

TYPICAL FALL SURVEYS OF HAY-FEVER FLORA

By O. C. DURHAM, KANSAS CITY, Mo.

GENERAL botanical works are too voluminous and too technical to be of practical use to the busy physician. A few excellent exhaustive hay-fever surveys have been made but they have served outside their immediate districts only as models. It is increasingly evident that local work must be done in every city of any size, but so far such work has been quite expensive. The accompanying table is the summary of the most important data obtained in a number of centers of population visited in one autumn. It is hoped that this report may point the way to effective pioneering in many places. There is always the temptation in this work to list every wind pollinated species encountered, but with the present mass of clinical data it is much wiser for the average physician to know and deal with a dozen locally important plants than to try to apply a mass of half reliable information.

By spending a few days in each place I was able to take physicians into the field and actually introduce them to their common important but often inconspicuous hay-fever weeds. Most of the work was done in waste areas in or very near the cities. The point will bear repetition that the cities are not only the weed breeders but also the residence of the great majority of pollen victims. In some of the places good work has already been done by local men but to make the paper as useful as possible my own observations are recorded.

In the chart only five groups are included. It has been found practical both in field and clinic to work with botanical groups. While specificity in test and treatment is highly desirable, many times group knowledge or grouping has saved the day. Trees are omitted on account of their relative unimportance. Cottonwood, oak, walnut and hickory are found in most of the cities listed and cause an occasional case. Mountain cedar is a real menace in the vicinity of Austin, Texas.

The grass data is not as complete as it should be for spring work. Bermuda grass and Johnson grass are abundant south of Kansas. Sudan grass is widely planted for hay and produces pollen much more freely than its close relative, Johnson grass. All three have long seasons of bloom especially in the south. Various species of Andropogon are widely distributed fall pollinators but their allergic importance has not been established. The composites, including goldenrod, sunflower (Helianthus spp), marsh marigold, broomweed and sneezeweed (Helenium) are common throughout the district visited but their clinical importance is small.

On account of their great importance the ragweeds should always be carefully noted. Short ragweed requires more moisture than false ragweed and western ragweed but less than giant ragweed, cocklebur, or marsh elder. The latter

will grow only on rich black lowland. Narrow-leaved marsh elder needs little water. Burweed marsh elder is the largest pollen producer of its genus but is not found south of Kansas City. It is common in eastern Colorado, Nebraska, and as far east as Chicago. Cocklebur should not be neglected.

DISTRIBUTION CHART
FALL SURVEYS

		St. Louis	Kansas City	Joplin	Wichita, Kans.	Denver	Oklahoma City	Dallas Texas	Austin, Texas	San Antonio	New Orleans	Shreveport, La.
RAGWEED GROUP												
Short Ragweed	Ambrosia elatior	*	*	*	*	*	*	*	*	*	*	*
Giant Ragweed	Ambrosia trifida	*	*	*	*	*	*	*	*		*	*
Western Ragweed	A. psilostachya	*	*		*	*	*	*	*			
Southern Ragweed	A. bidentata	*	*		*							
Cocklebur	Xanthium commune	*	*	*	*	*	*	*		*	*	*
Marsh Elder	Iva ciliata	*	*	*	*		*	*	*		*	*
Burweed Marsh Elder	Iva xanthiifolia	*			*							
Narrow-leaved M. E.	Iva angustifolia								*			
False Ragweed	Franseria acanthicarpa						*					
Bur Ragweed	Franseria tenuifolia									*	*	
CARELESSWEED GROUP												
Redroot Pigweed	Amaranthus retroflexus	*	*	*	*	*	*					*
Spiny Amaranth	Amaranthus spinosus	*	*				*	*			*	*
Palmer's Amaranth	Amaranthus palmeri							*	*	*		
Water Hemp	Acnida tamariscina	*	*		*		*	*	*			
GOOSEFOOT GROUP												
Lamb's Quarter	Chenopodium album	*	*	*	*	*	*	*				*
Russian Thistle	Salsola pestifer	*	*		*	*	*					
Burning Bush	Kochia scoparia						*					
Shad Scale	Atriplex canescens						*					
WORMWOOD GROUP												
Annual Wormwood	Artemisia annua	*										
Mugwort	Artemisia ludovic. et spp	*		*	*	*						
Pasture Sage	Artemisia frigida						*					
GRASS GROUP												
Blue Grass	Poa pratensis	*	*	*	*							
Orchard Grass	Dactylis glomerata	*	*		*	*						
Timothy	Phleum pratense	*	*		*							
Redtop	Agrostis palustris	*	*									
Bermuda Grass	Capriola dactylon						*	*		*	*	*
Johnson Grass	Holcus sorg. halapensis						*	*		*	*	*
Sudan Grass	Holcus sorg. sudanensis	*	*		*			*	*		*	

Careless weeds are found wherever there has been cultivation, spiny amaranth demanding very rich soil and water hemp very moist soil. The latter and Palmer's amaranth bear their pollen on a separate plant from that producing seed, while the ragweeds have flowers of both sexes on the same plant.

Lamb's-quarter is not as plentiful in Texas as in Kansas and Missouri and only when it has the advantages of cultivated land and plenty of moisture does it produce pollen abundantly. Russian thistle, *Kochia*, and *Atriplex* are not common except in Colorado where they rival the ragweeds in importance.

The wormwoods are dry prairie plants and while not abundant except in

the west of our district are quite toxic and should be taken into consideration wherever found.

The expense of these surveys has been largely met by Drs. W. W. Duke, Kansas City and R. B. Giles, Dallas. Thanks are due to the following men for their part in helping to make the work possible; Dr. R. M. Balyeat, Okla-
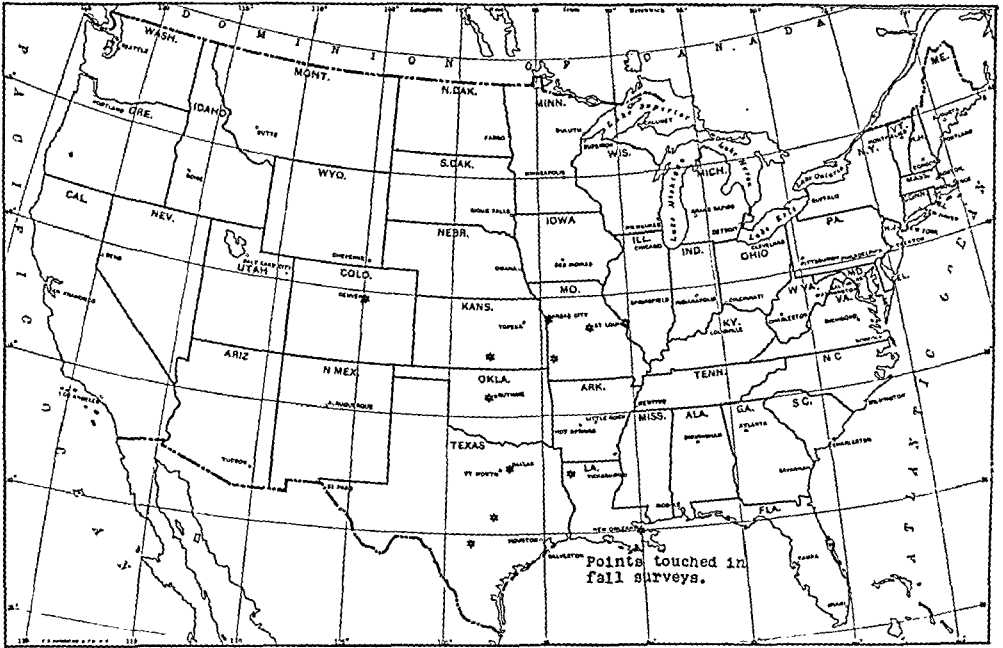
Chart I.

homa City, Dr. T. C. Terrell, Fort Worth, Dr. Chas. H. Eyermann, St. Louis, and Dr. W. C. Tennery, Waxahachie, Texas. Valuable botanical assistance was rendered by Mr. B. F. Bush, Courtney, Mo., Professor B. C. Tharp, Austin, Mr. Albert Ruth, Fort Worth, and Dr. Greenman, Missouri Botanical Gardens. The New Orleans data agrees with that published by Dr. Wm. Scheppegrell and is included merely for comparison.

THE LABORATORY IN DIAGNOSIS AND TREATMENT*

By WARD BURDICK, M.D., DENVER, COLORADO

THE age in which we live is marked by greater advancement in the arts and sciences than in all previous time. Medicine has kept the pace with progress in other departments of knowledge. Fifty years ago the world was practically in darkness as to the nature of the influences responsible for disease in the animal body, as well as the changes wrought in the tissues by these influences. Today the light of knowledge emanates from the scientific laboratories, and medicine, no longer a field of mystery, darkened by superstition, is so illuminated by the achievements of scientific research, that the practitioner of medicine of today is scarcely recognizable as the successor of the family doctor of yesterday.

The influence given to the practice of medicine from the scientific laboratories, has resulted in so broadening its scope, that graduates of medical colleges have ceased to follow the beaten path of precedent; but, like the fiery balls of a rocket, assume a separate course directed to the several fields of medicine, including surgery, urology, otolaryngology, internal medicine, tuberculosis, dermatology, neurology, pediatrics, etc.

The department of medicine which has received the distinction of clinical pathology embodies the practical application of the principles of gross morbid anatomy, pathologic histology, bacteriology, clinical microscopy, serology and chemistry. It, therefore, follows that there has developed a specialty in close relationship to the others and to which they turn, as to a mecca, for guidance. This branch penetrates to the recesses of all departments of medicine, and it is the purpose of this paper to review the various laboratory methods which are available as aids in diagnosis and treatment to the different phases of disease.

In order to relieve this presentation from the personal as much as possible, statements were solicited from physicians and surgeons who are prominent in their respective specialties. Thus, from the field of surgery come the views of Wm. J. Mayo,[1] who says: "As a general surgeon, I am glad to have this opportunity to express my obligation to the clinical pathologists, who have made possible one of the greatest advances in the surgery of the last decade. In the earlier days, the general surgeon, with the aid of the special senses, especially those of sight and touch, was engaged in gross surgery, usually of the destructive type, owing to the fact that the pathologic processes, of which

he was able to take cognizance, were advanced and, as a rule, indicated radical treatment. Today, through the aid of the various clinical laboratories, the surgeon works with the eye of the microscope, if one may so speak. Pathologic processes are discovered in the early stages of development, with the result that operative procedures can be followed when deviation from the normal physiology is least gross, and surgery has become constructive in its general tendency. The surgeon can be regarded as a means of mechanical therapy guided by the microscope, and for this guidance in the clinic we are indebted to MacCarty and Broders and their associates. For example, on opening the abdominal cavity to operate on a neoplasm, a small piece of tissue may be removed for microscopic examination and, within two or three minutes, not only is the nature of the growth known, but also, by the aid of Broders' index of malignancy, based on MacCarty's work on cell differentiation, the prognosis. If the cells are highly differentiated, the prospect of permanent cure is so great that one is justified in undertaking the serious type of operation; whereas, if the cells are largely undifferentiated, the prospect of cure is so small that a formidable operation would not be justified. Again, as in carcinoma of the large intestine or rectum, when enlarged glands occur, instead of assuming that the condition is incurable, a gland may be removed for microscopic examination. Often, enlarged glands are the result of sepsis, in which operation might possibly affect a cure. Not only is the work of the clinical pathologist important in the operating room, but also in the diagnostic clinic. The diagnosis in a given case may be in doubt. Before starting the patient on a long series of indefinite laboratory examinations which he may be in poor condition to withstand, it is often possible, under local anesthesia, to take a speciman of tissue from an enlarged gland in the groin or supraclavicular fossa, a thickened umbilicus, or from some other indicated situation, to be subjected to the acid test of a microscopic examination which tells truly the nature of the trouble and indicates the proper treatment for it.''

Regarding the value of clinical pathology as applied to Urology, Davis[2] writes that: ''Perhaps no surgical specialty is more dependent upon, or works more intimately with the laboratory, than does urology. The claim of urology to be regarded as a specialty, began with the development of intricate and difficult methods of diagnosis and treatment. The cystoscope, ureter catheter and the microscope have been closely associated in this accomplishment. The clinical pathologist and the roentgenologist have been partners with the urologist in this progress. As a result of this association and cooperation, I believe it may be fairly stated that urology today is one nearly exact art in medicine which latter is notoriously an inexact science. Much of this precision and exactness in urologic diagnosis and treatment is due to the laboratory. No competent genitourinary surgeon, today, would attempt an operation upon a kidney or a prostate, without first obtaining all the available data regarding the kidney and its fellow; or in the case of the prostate, without full knowledge of the combined renal function. The clinical pathologist has assisted in working

out the various tests for the determination of these facts—Ambard's constant, the estimation of the freezing point of blood and urine, phloridzin, methylene blue, and finally phenolsulphonephthalein, have been steps in this achievement. Tests of excretion and retention have been perfected. Blood chemistry—the estimation of retained nitrogen, nonprotein and urea, creatinine and other solids in the blood—is essentially a laboratory function. Of no less importance is bacteriology. Animal inoculation for diagnosis, notably in genitourinary tuberculosis, is done for us by the clinical pathologist, and in cases of suspected pyelitis, he confirms the diagnosis and tells us when our patient is cured. The Wassermann and other complement-fixation tests have been of inestimable value in diagnosis and treatment control; but may I here urge more investigation and research in the study of our little friend, the gonococcus. Cultural methods simplified or made more dependable and fermentation or fixation tests more satisfactory, are greatly needed to determine the cure of this widespread disease. In conclusion, however, I believe it may be truly said that the achievement of modern urology is due greatly to the exactness which the clinical pathologist and his laboratory have given to this specialty.''

The use of the laboratory in gynecology and obstetrics may be gleaned from the expression of Ingraham,[3] who avers that: ''No conscientious obstetrician in this day and age attempts to care for the pregnant woman without frequent routine laboratory examinations. We are dependent on the laboratory for the diagnosis and treatment of the various toxemias in pregnancy, and look to these workers for further advance as to the causes and classification of these not thoroughly understood abnormalities. There are many routine obstetric questions, such as, bacterial determination and tissue examination, which come up constantly. How little we would know of the physiology and the development of the ovum, if it were not for the laboratory. In gynecology, consider not very many years back, how frequent was the use of the curette, and the many diseases of the endometrium described. Through the work of Hitchmann and Alder, the various types of endometritis, have for the most part, been recognized as different stages of the menstrual cycle, and the curette is now seldom used except for diagnosis. The interrelation of the glands of internal secretion is probably more manifest in the study of gynecology and obstetrics than in any other branch of medicine. Much is expected from the clinical pathologist that these observations may be developed along practical lines. The pathologist has crystallized the subject of gynecology, so that today, lesions are recognized and classified and proper treatment instituted. As in every branch of medicine, gynecology and obstetrics are based on a sound knowledge of physiology and pathology for the proper understanding of these subjects.''

The clinical pathologist comes in contact with the public only upon invitation by the profession, as it were; his activities are carried on behind the scenes, a fact emphasized by Carmody[4] who reminds us that: ''While we are

[3]Ingraham, A. B., Ph.B., M.D.: Prof. of Obstetrics and Gynecology, University of Colorado School of Medicine.

[4]Carmody, T. E., M.D., B.D.S., D.F.C., F.A.C.S.: Past President of American Academy of Ophthalmology and Otolaryngology; Vice President of American Laryngological, Rhinological and Otological Society.

considering the value of different advances in medicine and surgery we most frequently think of what we see in the clinic room as of greatest importance, and while I do not wish to give the idea that marked improvement in technic of operating has not been found valuable, we should remember the man behind the lines as contributing much to the success of the operation. The clinical pathologist in the modern hospital is as necessary to the welfare of the patient as the internist or the surgeon, and many times saves us a false step. From the standpoint of the otolaryngologist the clinical pathologist may give valuable assistance in examining suspicious tumors at the time of operation, when, without his aid the patient might lose his larynx, or, if the growth should be malignant, an extensive operation may save his life. The same may be said of the accessory nasal sinuses. The Wassermann may solve the riddle of a persistent headache. The blood culture may help us to steer a course leading to success in suspected sinus involvement, either before or following a mastoid operation. Spinal puncture and the microscope may hasten interference on one hand or prevent meddlesome surgery on the other. These last-mentioned aids are to a great extent emergency in nature, but we must not forget the everyday routine examinations of blood, urine, and the various secretions and excretions, which may require hours of labor to point the way.''

Turning from the surgical specialties to the several branches of internal medicine we quote from Sewall,[5] who writes that: ''It seems but yesterday that the medical reactionary ventured to flaunt contempt upon the practical value of laboratory procedures, which now admittedly furnish essential material for the foundation of diagnoses. None will deny that certain examinations, as of the urine and of blood microscopy, should be performed as routine measures; for even superficial tests, if accurate, may be of intrinsic value or may lead to elaborate chemical analysis of the blood or investigation of renal efficiency. The clinical history or status of a case often demands that what may usually be regarded as an exceptional examination, become a routine measure—as the Wassermann test of blood and spinal fluid, a study of the blood pressures, blood grouping, or estimation of basal metabolism. The trained roentgenologist often demands information as to the clinical history of his subject before venturing to make a diagnosis from his x-ray findings. Still more important is it for the competent laboratory pathologist to become cognizant of the clinical status of the person under observation, in order not to lead astray the so-called clinician. The former should always be considered potentially, at least, as a consultant with the latter. The laboratory pathologist is neither a master nor a servant; but, as a matter of fact, we treat him as in the old days physicians were wont to treat the surgeon, as an operator who was bid to cut this or remove that with the aid of his trained technic. It is probable that many a diseased appendix has been allowed to rupture while the clinician, not trusting his own experience and common sense in interpreting indications has vacillated under the laboratory findings of casts and albumin in the urine, and fatally deferred operation until an expert could be found to catheterize the ureters. Frank discussion of the significance of such signs might save many lives. The

state of the patient as seen by the clinician takes precedence over any methodologic indications and the accumulation of 'scientific' data useless to the patient is dangerous pedantry. Besides a vast fund of learning, the laboratory staff should be able to put at our service, technicians meticulously trained in the performance of many procedures, such as the transfusion of blood, spinal puncture, etc. The laboratory man or woman thus comes in direct contact with the patient where applied psychology is of no little moment. The routine examination of tissues excised in the operating room not only may form the basis of valuable scientific deduction, but incidentally, it may serve to curb the operative fury of our colleague in whom the normal appendix excites, as it were, a persecution complex. In brief, it is my belief that, radiating from a narrow basis of routine examinations, the laboratory pathologist should be prepared to execute a far broader range of biologic investigations than is commonly in vogue, while no step should be taken unless the way is lighted by pathologic indications. For the perception of these indications, as in the interpretation of findings, the educated pathologist is an indispensable factor to the welfare of the patient. His duty does not end with the statement of his findings; he should be expected, in all appropriate cases to explain them and to point out their significance. This demands, at times, close study of the patient in cooperation with the clinician.''

As is well known, internal medicine is coming to be divided into subspecialties, as for instance the care of diabetics, the tuberculous, etc. From the realm of tuberculosis, Corper[6] expresses himself as follows: ''When asked to state my views on the laboratory in diagnosis and treatment in the few words allowed me for this purpose, I feel like the commanding officer who was given one hour's notice to embark for overseas service. The diagnostic laboratory in the clinic and the sanatorium is now so well established that it seems fair to take an inventory of, at least, a few of its important assets. I need not go far into the explanation of the examination of the sputum for tubercle bacilli, for all are well aware of the significance of a positive finding; whether obtained by examining the ordinary smear or by means of some of the more modern concentration methods accurately performed and under precautions to eliminate all possibilities of error. I dare say, however, there are few who fully recognize the value of this method in treatment. Admittedly we are aware of the fact that it is good practice to note whether our case is a persistent open case or one that has become closed, for much of the family's future welfare depends upon this; but it is only in the sanatorium where we can watch our cases year in and year out, that we appreciate all the possibilities in prognosis. We have passed that stage in our preliminary experiences when we believed tuberculosis incurable. It is true there are still some cases that come to us thus, but who wishes to judge absolutely that such is the case and who will not, now and again, change his views on further accurate study? Specifically our patient comes to us, he is at the present time not satisfied to know that he is tuberculous but he wants to know what the future has in store for him, and it is our obligation to give him the prognosis to the best of our

ability and with the aid of our most modern scientific weapons. Advisedly I call them weapons because too many of us stand off in fear and horror of them. I believe I can best illustrate my point by citing the conclusions of an article by Wingfield[7] and Wilson[8] of the Brompton Hospital Sanatorium, Frimley, in England, which just appeared in a recent number of the *Lancet*. They investigated the criteria for artificial pneumothorax in relation to treatment and state that among other indices the persistence of tubercle bacilli in the sputum after the induction of artificial pneumothorax is a feature of bad omen. Their figures showed that if the sputum remains positive the chances are nineteen to one against the patient being discharged as improved. Likewise we have learned that a great deal of information during treatment and in prognosis is obtained by means of the proper use of the urochromogen or diazo-reactions, repeatedly and conscientiously performed. They are an index of tissue destructive processes going on in the body, and as such, their persistence spells an unfavorable prognosis. I could mention others but refrain from doing so for lack of time. True you may say, but haven't we other means of determining the same points? Yes and no—Were we to give the time and effort, we might obtain the same information by other means, and yet frequently in the experience of the laboratory man, he has had to point out a plausible error on the part of the clinician and surgeon. It is human nature to err, yet our duty to the patient as physicians will not condone this, especially when we are able to make use of simple, economical and rapidly performed tests. An impending intoxication can frequently be discovered by chemical tests before it has reached serious and irremediable proportions. The slogan in tuberculosis which can well be applied to all other phases of medicine is: early correct diagnosis paving the way for early treatment, spells a good prognosis; and in this, the laboratory has developed instruments and methods of precision which are continually being perfected for speed and simplicity, besides being exhaustively evaluated by progressive students of our profession. Shall we stand back and ignore the beacon light of the times, or shall we still further increase the average span of human life, by treating with the appearance of the first sign of a pathologic condition, and when it is still in a remediable stage and can follow its course by means of rapid, delicate and accurate tests to restitution? The laboratory like the sanatorium has, by established ability, come as a permanent fixture; and those of us who do not avail ourselves of and become cognizant of both of these weapons of modern medicine are not fulfilling the requirements and duties placed upon us in accepting this noble profession as a life's work.''

Lingenfelter,[8] speaking with reference to the laboratory in the diagnosis and treatment of diseases of the skin quotes: "An old and true saying, which is, 'That, the better general practitioner one is, the better specialist he is likely to become.' We could equally well go a step farther and say, 'The more that he

[7] Wingfield. R. G., M.D., M.R.C.P.: Brompton Hospital Sanatorium; and Wilson, G. Selby, M.D., M.R.C.P., D.P.H.: Manchester.

[8] Wingfield, R. G., and Wilson, G. Selby: An Investigation into Criteria for Artificial Pneumothorax Serviceable as Guides to Treatment, Lancet, London, 1924, ccvii, 163.

[8] Lingenfelter, G. P., M.D.: Professor of Dermatology and Syphilology, University of Colorado, School of Medicine.

makes use of the facilities of the laboratory, the greater dermatological success will he enjoy.' It is much to be regretted that in studying the classification of diseases of the skin, one sees so frequently the word 'unknown' in the paragraph devoted to etiology. Particularly is this true of the malignant new growths, of tumors of the lymphatics and of the blood vessels. The laboratory gave us our first accurate knowledge of the cause of syphilis and paved the way to more successful treatment. It is to the laboratory that we must look for the cause and, eventually, the cure of cancer. To this department of medicine we are indebted for the knowledge of the causal agents in those diseases of the skin due to parasites and fungi. In this connection, special mention may be made of epidermophytosis which until within comparatively recent years, had been, all too frequently, wrongly diagnosed and treated. Many of the cases being confused with eczema, pompholyx, and dermatitis venenata. It is extremely possible that many cases went unrecognized. While Hippocrates affirmed that 'The body can only be understood as a whole,' thus hinting against the possible dangers of excessive specialism, we are also reminded of an aphorism of that estimable gentleman, Celsus, who, during the reign of Tiberius Caesar, wrote, 'It is not to be thought that he should know the remedies for disease, who does not know their original causes.' Thus, clinical observation based upon experience and supplemented by accurate laboratory work must eventually lead us to a more exact knowledge of the causes of, and the remedies for, many of the diseases of the skin which are now only partially understood.''

Perhaps no department of medicine requires closer cooperation with the laboratory than that of neurology. This is stressed by Moleen[9] and Bluemel[10] in the following communications. Says Moleen: ''As in other departments in medicine, the laboratory is a most important aid in the determination of the nature and location of nervous disease; but while approaching exactness, is still relative in value and, therefore, should never supersede clinical evidences. The determination of the blood reactions may often decide the presence or absence of inflammatory foci, and the careful quantitative estimation of urinary solids contributes to the establishment of renal insufficiency in cerebral arteriosclerosis and associated uremia. Examination of the spinal fluid may supply evidences of value in the explanation of pressure signs in the brain and spinal cord; and especially in trauma, will careful examination of this fluid often lead to definite conclusions. The most important association of the laboratory and neurology, however, is in that group of diseases which may differ widely in their clinical aspects, and yet, are attributable to the syphilitic virus, even though this virus may, with good reason, be suspected of significant morphologic and biologic variations. It is in the careful examination of the complement-fixation characteristics of the blood and spinal fluid which often makes a definite statement possible with reference to the clinical type of disease as well as to the prognosis. In the evolution of the various tests there has arisen considerable difference of opinion as to the value of each of the groups, such as the phases of Nonne or the

titration formula of Kolmer and others. The painstaking laboratorian has made the gold-sol reaction of Lange of inestimable value, especially in differentiating the vascular from the parenchymatous types of disease. Finally the most significant place for the laboratory is in the intensive treatment of cerebrospinal diseases, and especially syphilis. It is here that closest association between the neurologist and the clinical pathologist be encouraged. The intravenous or intraspinal treatment is best administered by the competent clinical pathologist. who can also supply, at the same time, evidences by means of which the clinician may draw helpful deductions; and thus may guide in the modification, continuance or discontinuance of the treatment in view of the composite formed by the laboratory findings and the clinical or bedside evidences. The only caution to be emphasized is the avoidance of the overestimation of relative reactions and the tendency under such circumstances to displace clinical facts by laboratory estimations. The value of the laboratory to the clinical neurologist is increasing not only as a diagnostic aid but in the prosecution of treatments. It is a branch of medicine which should be filled by trained and experienced men; and when so occupied, is entitled to the fullest measure of support and encouragement of the clinician. The nearer the cooperation of the laboratorian and the clinical neurolgist, the more prompt will be the determination of many nervous and mental problems which are still obscured by the mist of our ignorance.''

Bluemel[10] claims that: ''One of the weak points of neuropsychiatry is the fact that diagnoses are too often rendered in terms of anatomy rather than disease, the localization of the lesion being emphasized rather than the pathologic process. As a rule such diagnoses are of little value, for the important thing is the fact that the disease is syphilitic, or tuberculous—or whatever it may be— and not that the lesion is situated a millimeter and a third to the right of the optic chiasm. When the neuropsychiatrist makes generous use of the laboratory, these regional diagnoses become less conspicuous, and clinical diagnoses come to the foreground, as they should. To establish clinical diagnoses the neurologist must demand an irreducible minimum of laboratory work. This minimum should comprise a Wassermann test and a urinalysis. The Wassermann test is the more important of the two examinations by reason of the fact that about twelve per cent of neurologic patients are luetic. Where syphilis of long standing is demonstrated or suspected, the spinal fluid should be examined as well as the blood; for the blood may be negative while the spinal fluid is strongly positive. In acute neurologic diseases, especially those with such cerebral symptoms as torpor and rigidity, the laboratory may give the exact diagnosis. Arterial blood in the spinal fluid identifies a cerebral hemorrhage; an increase in white cells in the fluid indicates an inflammatory condition and the Gram stain or the Ziehl-Neelsen stain may demonstrate the organism. Here we have a diagnosis of precision, and no such accuracy of diagnosis can be obtained by the clinician who neglects laboratory aids. In other types of neurologic cases the diagnosis is often established by such laboratory methods as blood chemistry, leucocyte counts, differential counts, etc. A cranial nerve palsy may be due to a leucemic exudate, and nothing but a white blood count will definitely identify the underlying systemic disease. In cases of coma such as fall to a neurologist's.

service in a large city hospital, the diagnosis can often be rendered only through laboratory aids. The patient, being unconscious, is unable to give an account of himself, and the physical examination may not tell the full story. Here the laboratory can often solve the riddle by establishing a definite diagnosis of uremia, diabetes, cerebral hemorrhage, meningitis, etc. The clinical pathologist may render aid to the neurologist not only in diagnosis but also in the treatment of luetic cases. Even if the neurologist administers his own salvarsan and does his own spinal drainages or gives his own Swift-Ellis treatments; he will need the aid of the laboratory in checking his results, and such aid is of the highest value. The neurologist is perhaps more dependent on the laboratory than other specialists, and he can neglect the laboratory only by being a poor neurologist.''

In conclusion may we quote the viewpoint of a pediatrician as expressed by Amesse,[11] who claims that: ''Any open-minded survey of the progress of medicine during the past two decades would promptly disclose to surgeon and internist alike, the brilliant part played by the clinical pathologist in the development of the various medical specialties, and in their permanent establishment among the scientific bodies of the period. The advance of medicine may fairly be compared with that of the science of navigation, at first merely an art, tinctured with superstition and fantasy, resting on the uneasy basis of a false philosophy. With the dawn of civilization savage beliefs were dispelled, old wives' tales were mocked; the mariner grasped the rudiments of oceanography and learned to use his senses more and his imagination less. As the centuries went by, critical studies of the ocean currents, the tides and the winds were made, and these were exchanged among seafaring tribes, until finally, with the discovery of the compass, they could 'voyage dauntless on the sea,' and extend the domination of mankind throughout the world. The clinical laboratory is the compass of the physician. It enables him to sail fearlessly over hitherto uncharted waters; to invade wide areas closed to his predecessors, and to achieve conquests where only defeats were known. It has broadened and strengthened, sustained and stabilized the entire sphere of medicine and surgery, and has borne its full share in redeeming the tropical world from its age-old heritage of disease and death. The pediatrician acknowledges without question his dependence on the laboratory to assist with the numerous problems presented in his clinical work. For many conditions, the knowledge gained solely through this source becomes indispensable in the proper conduct of a case. For example, in diseases of the blood, differentiation, with resulting variation in prognosis and treatment, cannot be determined without the continued support of expert laboratory examinations. In the management of congenital syphilis, in the diagnosis of diphtheria, typhoid, malaria, dysentery, tuberculosis, diabetes and pyelitis, we become still further indebted. Within the past five years, investigators in research laboratories have accomplished, in preventive pediatrics alone, through the intensive study of scarlet fever, diphtheria, measles and poliomyelitis, enough to forever merit the encouragement and admiration of those dedicating their lives to the welfare of the young.''

11Amesse, John, M.D.: Instructor Pediatrics, University of Colorado, School of Medicine.

A STUDY OF TISSUE AUTOLYSIS IN VIVO*

I. Blood Changes: Physical and Chemical

By Edward C. Mason, M.D., and Edward C. Davidson, M.D.

Assisted by C. W. Matthew and P. B. Rastello

M ANN[1] and his coworkers, while developing the technic of hepatectomy, which has so greatly added to our knowledge of liver physiology, observed that a completely hepatectomized animal lived longer than an animal in which a small amount of liver substance remained free in the abdomen. The completely hepatectomized animals, when furnished sugar, lived as long as thirty-five hours, while animals having a piece of liver "the breadth of two fingers" free in the abdomen died within sixteen hours.[2] This last observation has greatly interested us since one of us has had the occasion to use a blood anticoagulant of hepatic origin ("heparin") and from its mode of preparation[3] it is reasonable to assume that the process is accompanied by marked liver autolysis. Although only 100 mg. of "heparin" was administered to each patient, such an amount frequently produced marked toxic symptoms. The degree of toxicity varying greatly with the different preparations.[4]

We were further interested to learn whether the autolysis in vivo of such an amount of liver tissue would liberate sufficient anticoagulant to be demonstrated by changes in blood coagulation. Whipple and Hurwitz[5] have reported a marked decrease in fibrinogen during experimental chloroform poisoning, while Conradi[6] has demonstrated that autolysis of various body tissues is accompanied by the production of a blood anticoagulant. Therefore, we have been interested to learn whether the reported decrease in fibrinogen was an actual decrease or only a result of autolysis such as doubtless accompanies chloroform necrosis.

After discussing these points with Doctor Mann, he has encouraged our study of these related phases of the problem.

METHOD

All operations were performed in the evening under aseptic conditions using ether anesthesia usually with a preliminary injection of morphine sulphate ¼ gr. and atropine sulphate $\frac{1}{100}$ gr., the animals having been fed eight to ten hours prior to operation. Briefly the operation consisted of clamping the portion of the liver to be excised, ligating, sectioning, weighing and returning the sectioned liver to the abdomen. Due to the ease with which it presents, the left lobe was usually selected. However, in a few animals the small lobe lying to the left of the lobe containing the gall bladder was used. The operation usually required

twenty to thirty minutes and the sectioned liver weighed 30 to 110 grams. In the animals where hemorrhage was thought possible a small sponge was left against the liver stump.

None of the animals showed signs of surgical shock and all were standing the following morning. The general appearance of such animals improved until within two to three hours before death, and frequently within an hour of death, their general appearance did not give warning of the impending death. Shortly before death (usually one-half to one hour) the animal lies flat on one side with all four legs extended and respiration becomes somewhat more labored. The animal approaches death without struggle except in the very terminal state when the head is drawn backwards and the chin is drawn downwards toward the neck, the terminal posture suggesting air hunger.

On postmortem examination the abdomen usually contains 100 to 300 c.c. of brown colored fluid but in no case did the fluid appear to be actual blood. A pseudofibrinous exudate is usually present, it being different from a true fibrinous exudate in that it is easily removed, leaving a smooth shining surface. A small amount of such exudate is usually present over the dome of the liver and often intestine loops are loosely adherent to each other. The intestines are generally hyperemic with marked congestion. The omentum is well wrapped about the free piece of liver; the omentum being markedly hemorrhagic and discolored. The picture is similar in many ways to that of peritonitis. However, in no case was there any indication of a septic peritonitis; and such peritonitis as is present, should doubtless be considered as chemical. Probably the most marked changes occur in the free liver tissue. It appears to have undergone a complete transformation, there being present no gross characteristics of liver tissue. The color is that of light chocolate while the feel is that of lung tissue, being spongy and gas containing. There is always a marked loss in weight of the sectioned liver ranging from six to forty grams.

A total of fifteen dogs was studied in which the following blood factors were determined: (1) Plasma Volume, (2) Serum Volume, (3) Fibrin Content, (4) Coagulation Time, (5) Amino Acids, (6) Sugar, (7) Uric Acid, (8) Urea, and (9) Nonprotein Nitrogen. For our observations, we found it best to take blood samples at the following periods: (1) at the beginning of the operation (8-9 P.M.), (2) the following morning (9-10 A.M.) and (3) before lunch. If the animal remained alive, another sample was taken about 6 P.M. and each subsequent, twelve to twenty-four hours throughout life. Of our series, two-thirds of the animals died in fifteen to eighteen hours, while two lived nearly seventy-two hours.

Plasma Volume Changes.—Plasma volume was determined through hematocrit determinations, all determinations being made in duplicate and all samples were centrifuged thirty minutes at the same rate of speed. The readings thus obtained are subject to a correction, since each ten c.c. of blood contained 1 c.c. of 1 per cent sodium oxalate solution. This source of error has been disregarded and the results have been expressed in per cent of oxalate plasma present. Chart I is presented to show the plasma volume before operation and at various periods following operation. Of the eight cases charted, it will be observed, all

show a marked decrease in plasma volume during the first twelve hours following operation. At the end of twelve hours the plasma volume either continued to decrease until the early death of the animal or soon showed an increase, and such animals lived almost seventy-two hours. While it is true two of the series showed a terminal increased plasma volume, this increase appears to be associated with the animals dying, as both samples were taken while the animals were expiring.

Serum Volume.—The lower portion of Chart I is presented to show the serum volume variations. The figures charted were obtained by allowing the blood to clot, after which the clot was broken and the tubes centrifuged as in the hematocrit determinations. The results, thus obtained, closely parallel those obtained in the plasma volume determinations, except that in some cases the
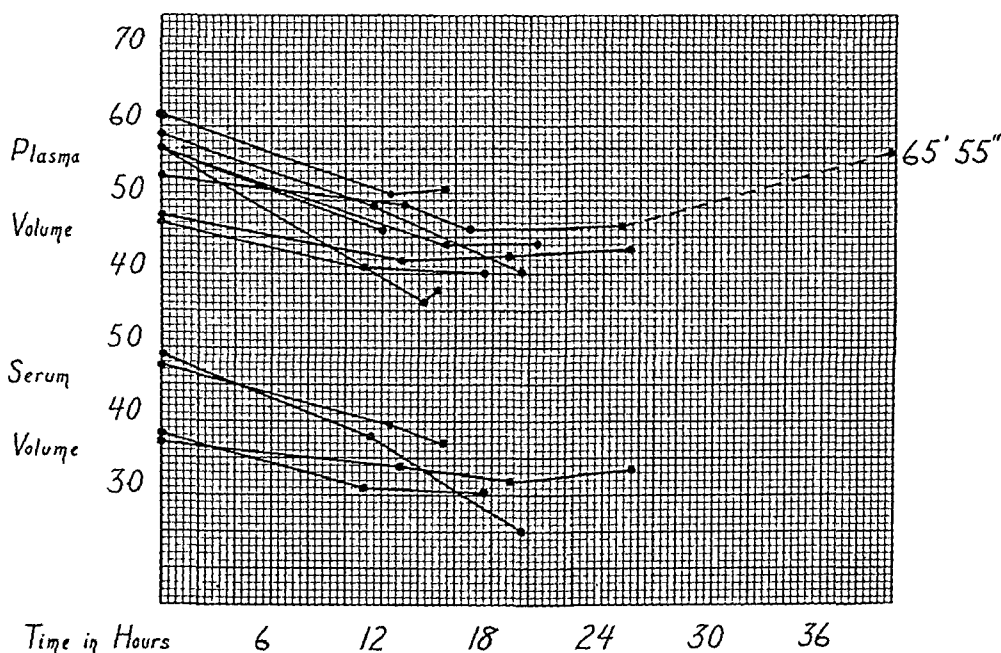
Chart I.

serum volume change is even more marked. The plasma volume in one animal changed from 59 per cent to 40.5 per cent, while the serum volume of the same animal changed from 49 per cent to twenty-five per cent. This observed change in fluid balance is apparently due to one or more of the following conditions: (1) decreased fluid intake, (2) vomiting, (3) increased hydration capacity of the tissues, (4) accumulation of fluid in the abdomen.

We did not have adequate facilities for making an accurate study of the urinary output but, from our observations, we believe that the animals do not become anuric and any lessened urinary output is doubtless associated with one or more of the above factors.

Fibrin.—Fibrin determinations were made according to the method of Foster and Whipple.[7] Briefly the method consists of recalcifying oxalate plasma, collecting the fibrin formed, drying, weighing, and igniting. The results are·

briefly shown in Chart II. In all cases studied, there was a definite fibrin increase, the increase being in some cases 100 per cent. On first thought, one might consider such an increase as being due to increased blood solids accompanying the decreased plasma volume. However, comparison of the plasma volume and the fibrin content shows the concentration of fibrin continues to increase even after the plasma volume begins to return to normal (demonstrated in the charts of the two animals living seventy-two hours).

Blood Coagulations.—Coagulation determinations were made using the Howell tubes. In the series of seven cases studied, all but one showed an increase in coagulation time during the liver autolysis. The average coagulation before operating was 15.8 minutes and the average of the last samples obtained
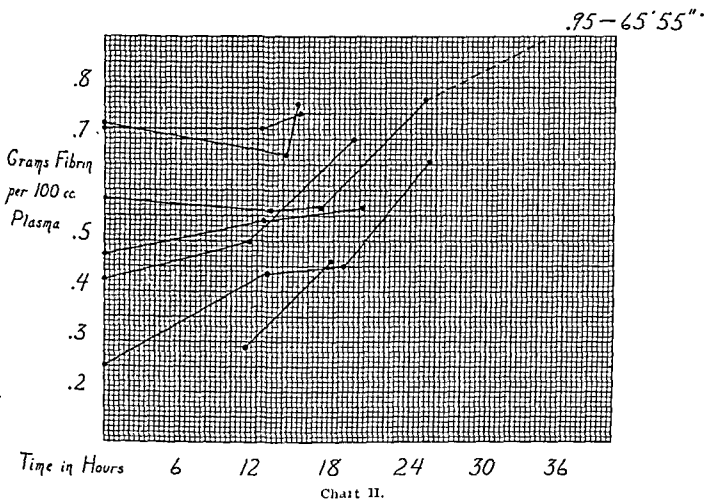
Chart II.

was 20.4 minutes. Therefore, it is suggested that autolysis of such an amount of tissue is accompanied by some delay in blood coagulation.

Amino Acids.—The Folin[8] method was used in the determination of amino acids. Chart III briefly shows the variations observed. From our findings it appears that there is a definite drop in blood amino acid nitrogen for the first period of approximately twelve hours, unless such a period ends close to the time of the animal's death. There also appears to be a very definite amino acid nitrogen increase beginning shortly before and continuing up until the animal's death. Considering the various nitrogen constituents as a possible source of toxic material which might cause the animal's death, it seems most reasonable to believe the amino acid portion most potent. It appears, however, if the toxicity is due to the amino acid fraction, that it must be a qualitative amino acid increase rather than a quantitative increase which proves fatal. This last statement is

supported by the two animals living seventy-two hours, in which case the amino
acid content showed some increase above normal without death of the animal.

Sugar.—Blood sugar determinations were made according to the method of
Folin and Wu; the blood filtrates being made immediately after the withdrawal
of the blood and sugar determination made either immediately or the filtrate
kept on ice until a satisfactory time for the determination. Chart IV shows
the usual type of sugar curve obtained. However, in one animal the sugar was
higher at the end of twelve hours than at the onset of the experiment; and in
another, it was higher at death than at the beginning of the experiment. We
are rather inclined to believe these two results may not be reliable and that the
curve presented in Chart IV represents what actually happens in blood sugar

Chart III.
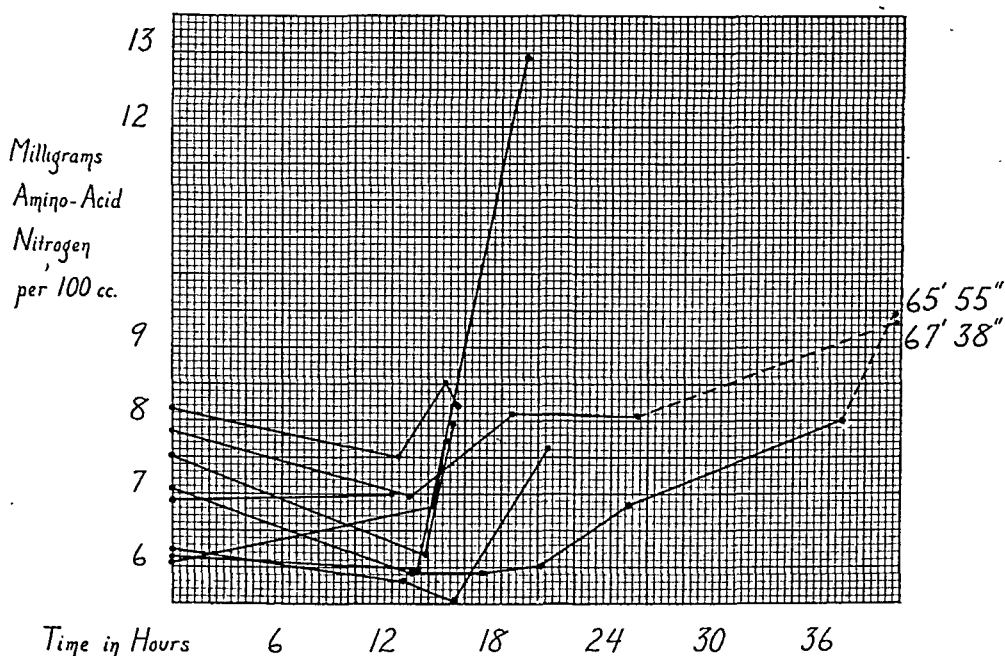
concentration. There appears to be a pronounced drop in blood sugar during
the first twelve hours, following which the continued drop is more gradual
until the early death of the animal. The two animals of our series which lived
nearly seventy-two hours showed a gradual increase in blood sugar following
the first period of eighteen to twenty-four hours. While the blood sugar drop
is quite marked, it is not sufficient to account for death of the animals. The
lowest level was 53 mg. per 100 c.c. blood.

Urea Nitrogen.—Urea determinations were made according to the method
of Van Slyke and Cullen. The results of such determinations are illustrated
by Chart V, the urea concentration becoming less during the first twelve hours
and rising rather abruptly before death. The curve is very similar to that
of the amino acids and, therefore, also to the nonprotein nitrogen curve. Al-
though the urea nitrogen curve is similar to the amino acid curve, it apparently

is not as good an index of the animals condition; for it will be noted that the amino acid fraction often shows a rise, while the urea fraction is still low.

Uric Acid.—Morgulis and Edwards[9] found uric acid to be 0.9 to 2.2 mg. per 100 c.c. in the normal dog. Hayden and Orr[10] found the amount of uric

Chart IV

Chart V.
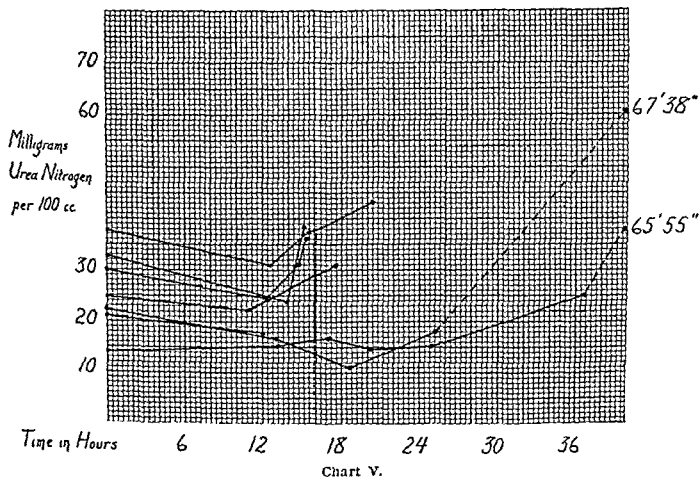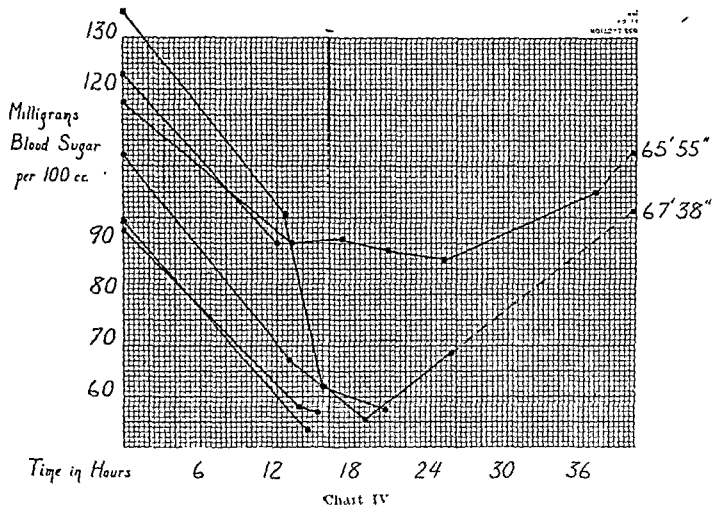
4. Blood studies show marked and constant changes. However, such changes do not account for the toxic element causing the death of such animals.

REFERENCES

1aMann, F. C.: Technic and General Effects of Removal, Am. Jour. Med. Sc., 1921, clxi, 37, also Am. Jour. Physiol., 1921, lv, 285.
1bMann, F. C., and Magath, T. B.: The Effect of the Removal of the Liver on the Blood Sugar Level, Arch. Int. Med., 1922, xxx, 73.
1cMann, F. C., and Magath, T. B.: The Effect of Administration of Glucose in the Condition Following Total Extirpation of the Liver, Arch. Int. Med., 1922, xxx, 171.
1dWilliamson, C. S., and Mann, F. C.: The Hepatic Factor in Chloroform and Phosphorus Poisoning, Am. Jour. Physiol., 1923, lxv, 267.
1eBollman, J. L., Mann, F. C., and Magath, T. B.: Effect of Total Removal of the Liver on the Formation of Urea, Am. Jour. Physiol., 1924, lxiv, 371.
1fMann, F. C., Bollman, J. L., and Magath, T. B.: The Formation of Bile Pigment After Total Removal of the Liver, Am. Jour. Physiol., 1924, lxiv, 393.
2Mann, F. C.: Personal communication.
Mann, F. C., and Magath, T. B.: Am. Jour. Physiol., 1922, liv, 486.
3Howell and Holt: Am. Jour. Physiol., 1918, xlvii, 328.
4Mason, E. C.: The Use of Heparin in Blood Transfusion, JOUR. LAB. AND. CLIN. MED., 1924, x, No. 3.
5Whipple and Hurwitz: Jour. Exper. Med., 1911, xiii, 136.
6Conradi: Hofmeister's Beitr., 1901-1-136. Beitr. z. chem. Phys. u. Path., 1902, 1, 136.
7Foster and Whipple: Am. Jour. Physiol., 1922, lviii, 366.
8Folin: Lab. Manual of Bio. Chem., D. Appleton & Co., New York and London, 1922.
9Morgulis and Edwards: Am. Jour. Physiol., 1924, lxviii, 477.
10Hayden and Orr: Jour. Exper. Med., 1923, xxxvii, 365; xxxviii, 477.
11Draper: Jour. Am. Med. Assn., 1917, lxix, 1768.

II. THE POINTS OF ACTION OF SODIUM PHENOBARBITAL AND PHENOBARBITAL IN LOWERING BLOOD PRESSURE*

By CHARLES M. GRUBER, PH.D., M.D., AND ROY F. BASKETT, M.D., ST. LOUIS

IN the twelve years since the introduction of luminal and luminal sodium a great deal of work has been done on their uses in the treatment of epilepsy and as hypnotics. Even with the vast number of such cases treated and the consequent wealth of material to work upon no one seems to have noted how or where these effects are accomplished. The literature on the subject yields only three investigators who mention their possible effect on blood pressure.

In 1912, Impens[1] noted that luminal and luminal sodium were able to lower the blood pressure in experimental animals, and in some cases produced an additional rise. Wetzel,[2] after failure to find any effect upon the circulation when these drugs were given to experimental animals in hypnotic doses, made note of the fact. Gregor[3] observed that luminal lowers the blood pressure in human beings.

Recently we have shown[4] that sodium phenobarbital and phenobarbital increased the blood pressure twice in over one hundred and sixty-four injec-

tions in thirty-two dogs and nine rabbits. This subsequent investigation was undertaken to determine the effect of these drugs upon 1, the heart; 2, the volume of the limb, kidney, intestine and spleen; and 3, to note whether the change in the volume of the organs is due to the central or the peripheral action of these drugs, in other words, the point of action.

METHOD

Dogs, cats and rabbits were used. The dogs were anesthetized with ether by inhalation or with paraldehyde (1.8 ml. per kilo per os). The cats and rabbits were anesthetized with urethane 2 gm. per kilo body weight by stomach. After anesthetization the animals were tracheotomized.

In all the experiments, except those in which perfusion was employed, the blood pressure was taken from a cannula inserted in the left carotid artery and connected with a mercury manometer. A two per cent sodium
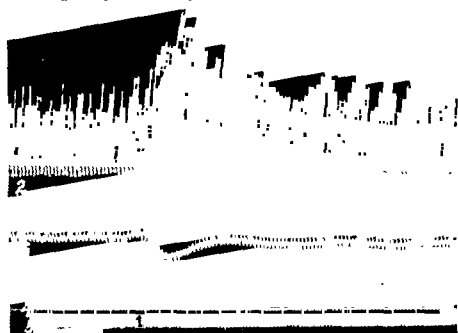
Fig. 1.—Cat, 3 kilograms, urethane anesthesia. Artificial respiration. Heart plethysmographed. 2, Record of heart; 3, blood pressure; 4, time in fifteen seconds and zero blood pressure and 5, time and duration of injection of drug. 1, 0.2 gm. sodium phenobarbital dissolved in 2 ml. Ringer's solution injected into the external jugular vein. Up stroke diastole, down stroke of lever systole.

citrate solution was used as an anticoagulant. A chronograph marking either five, fifteen or thirty second intervals was placed at the zero blood pressure level. The writing points were placed one above the other.

The organs of the abdomen were exposed through a midline incision. For plethysmographing a loop of intestine, a kidney or the spleen, metal oncometers were used connected with piston recorders which marked the changes in volume upon the drum surface. These were applied with as little manipulation of the organs as possible and the abdomen then sewed up. A metal leg oncometer connected to a piston recorder was used in recording changes in the volume of the hind limb. In all instances before the oncometer was applied the limb was disjointed at the ankle, so that the foot was not used in the plethysmograph oncometer. The limb and at least one other organ were always plethysmographed simultaneously and in many cases

the volume changes of three organs were recorded at once in addition to the recording of blood pressure.

After establishing artificial respiration the changes in heart volume were recorded by placing a glass cardiometer about the heart, exposed by excising the ventral half of the thorax with the loss of as little blood as possible. Changes in blood pressure were recorded simultaneously. In a number of instances records of both auricular and ventricular contractions were made simultaneously with cardiographs[5] applied directly to the heart.

In a number of cats the kidneys, loops of intestine and hind limbs were perfused. The perfusion method was essentially the same as that used by Sherrington and Sowton.[6] The free end of the arterial cannula was connected to a short rubber tube leading from a reservoir containing a Ringer's solution, modified by adding defibrinated blood from the animal. It was kept in a water-bath at 38.5° C. and oxygenated by passing air through it. The perfusion pressure varied from 60 mm. of mercury to 100 mm. but was kept practically uniform throughout each experiment, maintained by the rate of flow of air bubbling through the perfusion fluid. The excess air was allowed to escape through a mercury valve which supported the desired pressure. During the escape of air through the valve the pressure would fall 4 mm. of mercury. This variation occurred about every five to ten seconds. Sodium phenobarbital dissolved in the perfusion mixture 0.1 gm. per ml. was injected into the perfusate near the arterial cannula. Such injections had no effect upon the perfusion pressure as this was equalized by the escape of air through the mercury valve.

The drops of perfusion fluid, flowing from a glass cannula placed in the vein leading from the organ, were recorded on the kymograph by means of a receiving and a recording tambour.

The effect upon urine secretion was noted in a number of cases. The flow was recorded by placing metal cannulas into the ureters. Sodium phenobarbital* was always injected, except as previously stated, dissolved in Ringer's solution, 0.1 gram per ml. Phenobarbital* in the few instances studied was dissolved in ethyl alcohol 0.1 gm. per ml. alcohol.

In the cats and dogs these injections were made directly into the exposed femoral vein. In the rabbits the drugs were injected into the lateral ear vein.

<center>RESULTS</center>

The Effect upon the Intact Heart Muscle.—If the blood pressure records are studied closely it will be noted that the heart rate was slowed in all records in which large doses were used. The average slowing of heart rate in dogs by 0.1 gm. sodium phenobarbital was 6.1 per cent; in the rabbits the same dose caused a slowing in heart rate of 8.1 per cent. A slowing equivalent to that produced in dogs by 0.1 gm. was produced in rabbits by 50 mg. of sodium phenobarbital. In the dogs 0.2 gm. sodium phenobarbital decreased the rate 13.3 per cent, and 0.4 gm. caused an average slowing of 28.8 per cent. As this effect was not influenced by the cutting of the vagi,

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*The preparations used were presented to us by the Winthrop Chemical Company.
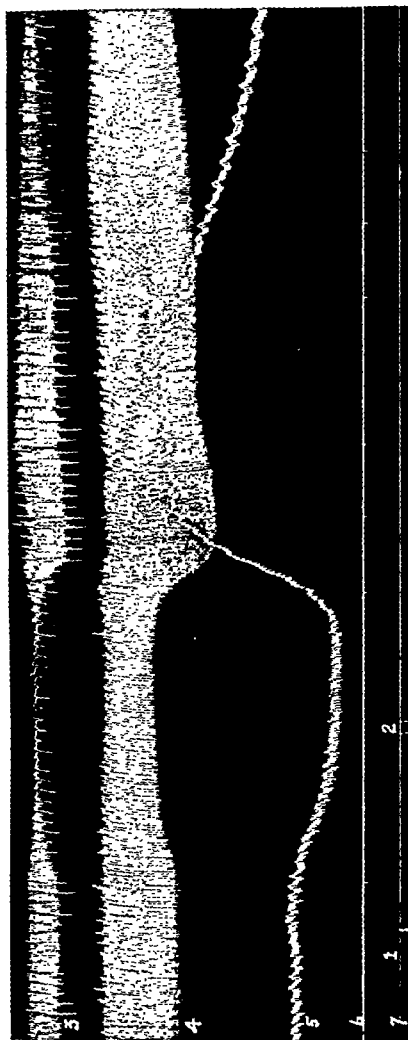
Fig. 2.—Dog, 14 kilograms, paraldehyde anesthesia. Artificial respiration. Up stroke of heart lever diastole, down stroke systole. 3, auricles, 4, ventricles, 5, blood pressure; 6, time in fifteen seconds and zero blood pressure and 7, time and duration of injections. 1, 0.4 gm. sodium phenobarbital dissolved in 4 ml. of Ringer's solution; 2, 1 ml. adrenalin chloride 1:10,000 solution.

the question therefore arose as to what effect sodium phenobarbital and phenobarbital may have upon the heart muscle itself.

The hearts of both cats and dogs were plethysmographed. It was noted that small doses, 25 mg. in cats and 50 mg. in dogs, had no effect upon the heart volume. Fifty mg. in cats and 0.1 gm. in dogs, when injected intravenously, caused an increase in the volume of the heart, i.e., the heart dilated. Larger doses produced further dilation but in our experiments never caused cessation of heart beat. Fig. 1 is presented as typical of all such experiments. A cat weighing three kilograms was anesthetized with urethane. The thorax was opened and the heart, exposed under artificial respiration with as little loss of blood as possible, was placed in a glass oncometer and the contractions recorded. The down stroke shows the systolic and the up stroke the diastolic contraction, any change in the curve upward indicates increased volume of the heart. At 1, 0.2 gm. sodium phenobarbital was injected into the external jugular vein. There resulted immediately a fall in blood pressure accompanied by an increase in the volume of the heart. Similar changes were noted in both cats and dogs.

The effect of these drugs upon the contractile power of both the auricles and ventricles was also studied. Small doses had no effect upon either the auricles or ventricles; larger doses caused practically complete cessation of contraction of the auricles and marked decrease in the length of the excursions of the ventricles and also a decreased rate of beat. (See Fig. 2.) In Fig. 2, a dog weighing 14 kilograms was anesthetized with paraldehyde and cardiographs were attached to both auricles and ventricles. At 1, 0.4 gm. sodium phenobarbital dissolved in 4 ml. Ringer's solution was injected into the femoral vein. Shortly thereafter the auricles ceased beating, the ventricles continuing to beat but at a much slower rate and with decreased force. Sodium phenobarbital, like most drugs, was found to be more toxic to the auricles than to the ventricles. Upon hearts so depressed we tried the effects of various so-called cardiac stimulants. Large doses of camphor, 20 ml. of a saturated solution, in Ringer's were rapidly injected intravenously without effect. Likewise 8 mg. strychnine sulphate were injected intravenously without effect. Adrenalin chloride on the other hand injected intravenously always markedly increased the force and rate of contraction. In Fig. 2 at 2, 1 ml. of a 1:10,000 solution of adrenalin was injected intravenously after section of both vagi. An immediate escape of the heart from the action of sodium phenobarbital was noted. The reverse condition will be seen in Fig. 3, in which the same animal was used, the adrenalin chloride being injected before the sodium phenobarbital. In this instance 1 ml. of a 1:10,000 adrenalin chloride solution was injected at 1 and 0.4 gm. sodium phenobarbital dissolved in 4 ml. Ringer's solution was injected at 2. The sodium phenobarbital even in the presence of adrenalin was able to slow the rate and decrease the force of the heart beat. Similar results were obtained with phenobarbital. It must be admitted that a part at least of the fall in blood pressure may be due to the effect of sodium phenobarbital and phenobarbital upon the heart muscle itself.
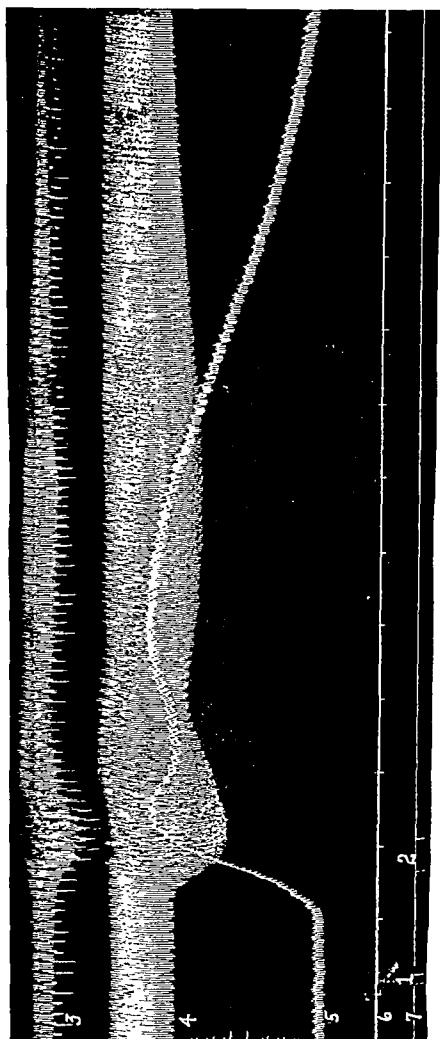
Fig. 3.—Same as Fig. 2, except at 1, 1 ml adrenalin chloride 1·10,000 was injected and at 2, 0.1 gm. sodium phenobarbital was administered.

The Effect of Sodium Phenobarbital upon the Volume of the Limb, Kidney, Spleen and Intestine.—In order to determine whether or not the fall in blood pressure brought about by sodium phenobarbital injection is the result of a weakened myocardium only, or whether the vascular system is also involved, the limb, kidney, spleen and intestine were plethysmographed to see if any
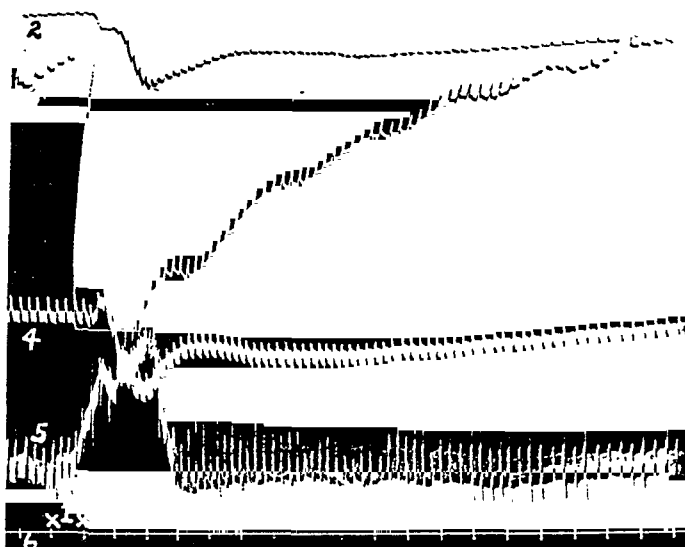
Fig. 4.—Dog, 12 kilograms, paraldehyde anesthesia, artificial respiration. *2*, Record of volume of kidney; *3*, volume of spleen, and *5*, volume of limb. *4*, Blood pressure record; *6*, time in fifteen second intervals and zero blood pressure. *1*, 0.2 gm. sodium phenobarbital injected intravenously. The blood pressure wrote 8 mm. in advance of all plethysmograph writing points. Up stroke of plethysmograph record indicates increased volume of organ, down stroke decreased volume of organ.
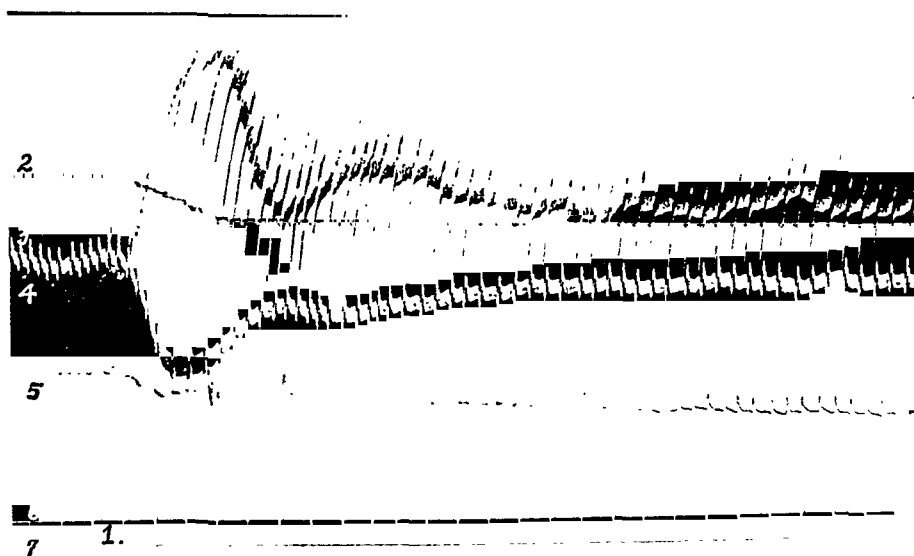
Fig. 5.—Dog, 16.4 kilograms, paraldehyde anesthesia. *2*, Record of volume of kidney; *3*, blood pressure; *4*, volume of limb; *5*, volume of intestine; *6*, time in fifteen second intervals and zero blood pressure, and *7*, point and duration of injection of drug. *1*, 0.2 gm. sodium phenobarbital injected intravenously. Up stroke increase in volume, down stroke decreased volume of organs.

change in volume occurred during the fall in blood pressure. In the animals so studied with two exceptions, sodium phenobarbital produced an increase in limb volume and a decreased volume of the spleen, of the kidney and usually of the intestine. In two animals the kidney increased in volume and the limb decreased with all the injections. Figs. 4 and 5 show the usual results. In Fig. 4, the blood pressure and the volumes of the kidney, spleen and limb were recorded. It will be noted that the kidney and spleen markedly diminished in volume while the limb increased. A similar condition is seen in Fig. 5. In this case the kidney and intestine decreased in volume simultaneously with a fall in the blood pressure and marked dilation of the limb. The dog in Fig. 4, weighed 12 kilograms and was under artificial respiration; the one used in Fig. 5, weighed 16.4 kilograms. Both animals received 0.2 gm. sodium phenobarbital dissolved in Ringer's solution intravenously at 1.
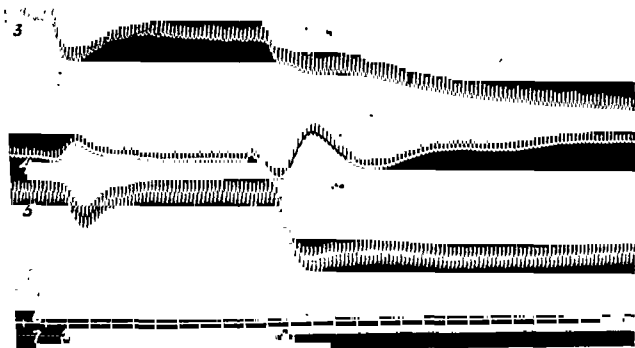
Fig. 6.—Same as Fig. 4. 3, volume of kidney; 4, volume of limb; 5, blood pressure; 6, time interval in fifteen seconds and zero blood pressure, and 7, point and duration of injection. 1, 2 ml. ethyl alcohol injected intravenously. 2, 2 ml. ethyl alcohol in which has been dissolved 0.2 gm. phenobarbital injected intravenously.

Fig. 6 is presented to show the effect of phenobarbital upon the volumes of the kidney and limb. The results are similar to those obtained with sodium phenobarbital. The same animal from which Fig. 4 was made was used, artificial respiration being continued throughout the experiment. At 1, 2 ml. ethyl alcohol were injected intravenously and at 2 an equal amount of alcohol containing 0.2 gm. phenobarbital. In 2, a temporary decrease in limb volume is noted, due to the sudden fall in blood pressure (passive constriction) but this is quickly compensated for, by an active increase in volume. The kidney decreased in volume after each injection. Both kidney and limb volumes returned to normal later, which fact does not appear in the record. Active dilation of the vessels of the limb seems to result from administration of sodium phenobarbital and phenobarbital.

The Effect of Sodium Phenobarbital Injected into the Perfusate upon the Vessels of the Excised Limb, Kidney and Intestine.—In order to determine if sodium phenobarbital has a peripheral action upon the vessel wall, experiments were performed in which the limb, kidney and loops of intestine were perfused with Ringer's solution containing defibrinated blood. The sodium phenobarbital was dissolved in the modified Ringer's solution and was injected into the perfusate through the rubber tubing near the arterial cannula. Figs. 7, 8 and 9 are records showing the effect of such injections upon the rate of flow of the perfusion fluid from the limb, kidney and intestine respectively. In Fig. 7, the gracilis, adductor femoris and semimembranosus muscles of the cat were perfused through the ramus muscularis branch of the femoralis artery[7] at a pressure of 60 mm. of mercury. At 1, 0.2 gm. sodium phenobarbital dissolved in 2 ml. blood Ringer's solution was injected into the perfusate. The rate of flow increased suddenly from 22 to a maximum of 58 drops per thirty seconds or an increase of 164 per cent. These results were unvariable. The blood vessels of the limbs of two animals simi-
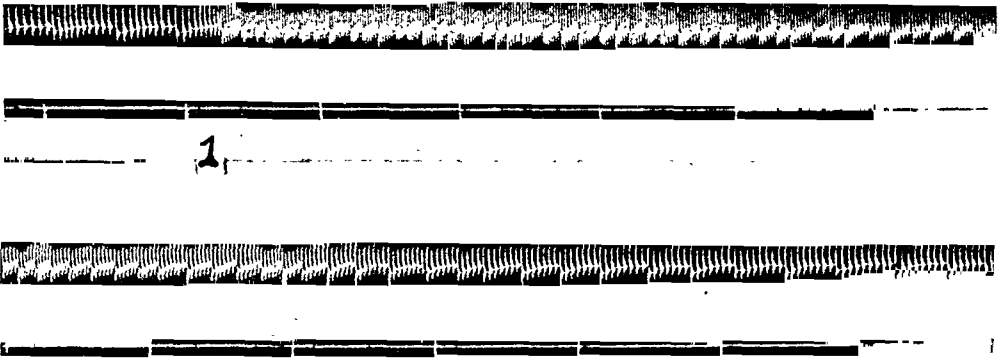
Fig. 7.—Muscles of a cat perfused with 38.5° C. Ringer's solution containing defibrinated blood. Perfusion pressure 60 mm. of mercury. Top record rate of flow of fluid from the muscles in drops. Middle record time in thirty second intervals. Bottom record, at *1*, 0.2 gm. sodium phenobarbital dissolved in 2 ml. of the perfusion fluid was injected into the perfusate.

larly treated, responded by a decreased flow of the perfusion fluid. The effect upon the kidney vessels differed from that upon the vessels of the limb. In Fig. 8, a cat's kidney was perfused at 90 mm. of mercury pressure. At 1, 0.2 gm. sodium phenobarbital was injected into the perfusate. There was an immediate increase in rate of perfusion flow, from 26 to 32 drops per thirty seconds which quickly decreased to ten drops for the same period. This vasoconstriction was followed later by a prolonged vasodilation in which the maximum rate of flow was 50 drops per thirty seconds, an increase of almost one hundred per cent. This change in blood flow with large doses was quite uniform throughout the experiments. The first increase was not due to increased pressure resulting from the injection but was due to the effect of the drug itself.

The results from perfusing the loop of intestine were uncertain. Usually

a decreased rate was observed. Fig. 9 is one in which a definite constriction was obtained. One-fifth gm. sodium phenobarbital dissolved in 2 ml. solution was injected into the perfusate. The rate of flow immediately decreased from 70 to 30 drops per thirty seconds or a decrease of 57 per cent.

The Effect of Sodium Phenobarbital upon the Secretion of Urine.—Although phenobarbital has an effect upon the kidneys, in the few animals tested it appeared to have no direct action on urine secretion. Fig. 10, is a record from a 14 kilogram dog in which sodium phenobarbital 0.3 gm. was injected at 1. There was a temporary cessation in the secretion due, we believe, to the fall in blood pressure and not to a direct effect upon the kidney. How-
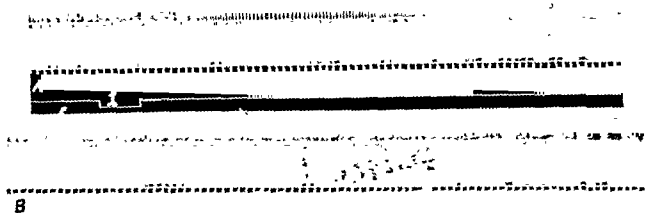
Fig. 8.—Cat's kidney perfused with 38.5° C. Ringer's solution containing blood. Perfusion pressure was 90 mm. of mercury. Top record flow of fluid from the kidney in drops; middle record time in five second intervals and bottom record, at 1, 0.1 gm. sodium phenobarbital was injected into the perfusate. B, continuation of A.
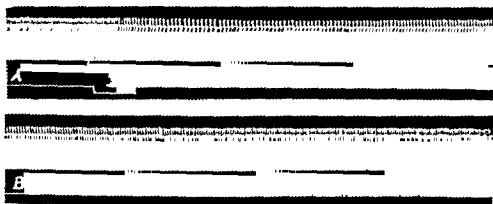
FIG. 9.—Loop of cat's intestine perfused with Ringer's solution containing blood. Perfusion pressure was 90 mm. of mercury and temperature of the fluid 38.5° C. Top record flow of fluid from loop of intestine in drops, middle record time in thirty seconds, bottom record at 1, 0.2 gm. sodium phenobarbital was injected into the perfusion system. B, continuation of A.

ever, in those cases in which the blood pressure remained permanently low after repeated small injections or after the injection of a large dose, the secretion of urine was usually diminished and in some cases anuria resulted. This again we believe to be due to the effect of sodium phenobarbital upon the blood pressure and not upon the kidney. Although sodium phenobarbital in large doses as in perfusion experiments is capable of causing vasoconstriction of the vessels of the kidney, it does not seem possible that when given by mouth it can be sufficiently concentrated to interfere with the urine secretion.

DISCUSSION

Many of the earlier writers believed that the heart rate was accelerated under the influence of phenobarbital and sodium phenobarbital. With our results on experimental animals we have been unable to confirm this. We found as the usual effect that the heart rate is slowed, the heart's force is diminished and that the heart itself is dilated slightly when acted upon by sodium phenobarbital. There appears to be a greater depression of the cutaneous vessels than of the visceral vessels and that the vessels of the limb dilate while those of the intact intestine, kidney and spleen constrict. How-
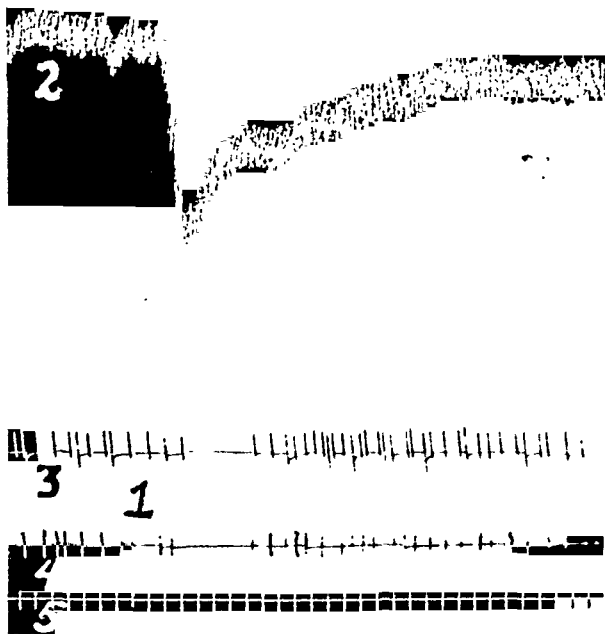
Fig. 10.—Dog, 14 kilograms, paraldehyde anesthesia. Top record blood pressure with mercury manometer, bottom record 5, time in fifteen second intervals and zero blood pressure. Records 3 and 4 are the rate of flow of urine from the left and right kidneys respectively. 1, 0.3 gm. sodium phenobarbital injected intravenously.

ever, the perfusion experiments show that the vessels of the kidneys may also dilate and those of the limb may constrict.

SUMMARY

1. Phenobarbital and sodium phenobarbital, when injected intravenously, cause slowing of the heart rate and decrease of the force of the heart beat.

2. Phenobarbital and sodium phenobarbital, injected intravenously cause slight dilation of the heart.

3. Phenobarbital and sodium phenobarbital, given intravenously usually decrease the volume of the spleen, kidney and loop of intestine, but cause an increase in volume of the limb.

4. Perfusion experiments of the kidney, limb and loop of intestine show the action of sodium phenobarbital to be peripheral and not central in origin.

5. Perfusion experiments on the kidney and limb show that sodium phenobarbital may cause dilation of the vessels in these organs as well as constriction.

6. Sodium phenobarbital in moderate doses has no direct action upon the secretion of urine, although if given in large doses the resulting fall in blood pressure is capable of decreasing the urine secretion even to the point of causing anuria.

REFERENCES

[1]Impens: Deutsch. med. Wchnschr., 1912, xxxviii, 945.
[2]Wetzel: Berl. klin. Wchnschr., 1912, xlix, 937.
[3]Gregor: Therap. Monatsch., 1912, xxxvi, 418.
[4]Gruber and Baskett: Jour. Pharmacol. Exper. Therap., 1925
[5]Jackson: Experimental Pharmacology, St. Louis, 1917, p. 149.
[6]Sherrington and Sowton: Brit. Med. Jour., 1903, Supplement cxlvii.
[7]Reighard and Jennings: Anatomy of the Cat, New York, 1901, p. 310.

SYNERGISTIC ANALGESIA AND ANESTHESIA WITH SPECIAL REFERENCE TO MAGNESIUM SULPHATE, ETHER, MORPHINE AND NOVOCAINE*

By James T. Gwathmey, M.D., and Charles W. Hooper, M.D., New York City

THE late Samuel J. Meltzer entertained and defended the view that the phenomena of life are not simply the outcome of a single factor of excitation, but that they are the results of a compromise between two antagonistic factors, the fundamental forces of life—excitation and inhibition. In a series of experiments on the effects of intracerebral injections of solutions of various salts carried out in 1898, Meltzer observed that an intracerebral injection of two drops of a 5 per cent solution of magnesium sulphate produced complete anesthesia and relaxation of the animal lasting several hours. On the basis of the hypothesis that magnesium is an inhibitory factor in the life phenomena, this observation led to a prolonged experimental study of the effects of magnesium salts on the animal body and to many publications on this subject. In their first publication in 1905, Meltzer and Auer made the general statement that the salts of magnesium are capable of inhibiting the entire nervous system, and assumed in particular that these salts are also capable of producing anesthesia and other inhibitory effects on the central nervous system. They found that after an injection of a proper dose of a solution of magnesium sulphate the animal loses for some time all reflexes and signs of sensibility, while the respiration remains intact. This first publication led to an extensive literature on the subject. Some of the writers insisted that magnesium exerts only a curare-like action, that is, the salts

paralyze the motor nerve endings to such a degree that the animal is incapable of responding to any stimulation; in other words, that the animal which appears to be anesthetized is actually conscious during the operation and feels all the pain inflicted on it, but is incapable of manifesting its sensations on account of the complete paralysis. Meltzer and his coworkers labored for many years to establish magnesium sulphate as an anesthetic agent. They investigated the effect of magnesium salts on animals and in a comparatively considerable degree also on human beings by different modes of administration: by intravenous, intraspinal, intramuscular and subcutaneous injections. In all modes of administration the effect was found to be unmistakably depressing in character. They found among other things that the hypodermic injection of a concentrated solution of magnesium sulphate had a profound depressing effect upon the nervous system without any preceding excitation. The subcutaneous injection of magnesium sulphate never led to an immediate or late appearance of diarrhea or more frequent stools. The salt is eliminated to a great extent through the kidneys. They further observed that calcium antagonizes the abnormal activity of its three inorganic associates in the animal body, sodium, potassium and magnesium, be the activity an over-inhibition of an over-excitation.

In 1916, Peck and Meltzer employed magnesium sulphate clinically as an anesthetic in man. These observations proved conclusively that the state of anesthesia which is produced by injection of magnesium sulphate is actually anesthesia, that is, in this state sensation as well as consciousness are temporarily abolished. They found that the anesthesia may or may not be accompanied by a pronounced paralysis of the endings of the motor nerves of a great part of all skeletal muscles. The central effect, especially the effect on the sensation of pain and on consciousness, can be attained with a smaller dose of magnesium sulphate than that which is required for a paralysis of the motor nerve endings. The central effect also appeared to set in sooner than the peripheral one.

I. SYNERGISTIC ACTION OF MAGNESIUM SULPHATE AND ETHER

Meltzer and Auer, 1913-14, made the surprising observations that after the intramuscular injection of one-half or less of the anesthetic dose of magnesium sulphate, rabbits and dogs are readily and deeply anesthetized by inhalations of small doses of ether which are insufficient to anesthetize normal animals. They found the anesthetic dose of magnesium sulphate by intramuscular injection to be about 1.2 grams per kilo for the rabbit and between 1.75 and 2 grams per kilo for the dog. In the experiments which they reported, rabbits were given 0.6 gram (or less) and dogs 0.8 gram (or less) per kilo; with these small doses the animals were only slightly affected; if ether was insufflated eight or ten minutes after such an injection, the animals became deeply anesthetized. The dose of ether which was sufficient to cause deep anesthesia was hardly a tenth of the dose which was otherwise required for a similar deep anesthesia without the help of magnesium sulphate. Each experiment consisted of a series of three animals; for example, one dog

received 0.8 gram magnesium sulphate per kilo, another inhaled a small dose of ether, and the third inhaled an equal amount of ether after it had been injected with 0.8 gram magnesium sulphate per kilo. The ether was administered simultaneously to the second and third animals by means of cannulae of equal size from the same ether flask. The first and second animals were only slightly affected. The third animal always became deeply anesthetized, and the course of the anesthesia was as follows: if a stream of air containing about 3 per cent ether was administered, deep anesthesia developed after a few minutes, in which the abdomen or pleural cavity could be opened, the parietal peritoneum or parietal pleura scratched and the sciatic nerve exposed, taken up on a thread and torn, without a sign of pain sensation or other reaction. In the first stage the lid reflex remained quite prompt and sometimes short, apparently spontaneous winking motions occurred; irritation of the central end of the vagus caused cessation of breathing which was proceeding regularly; irritation of the sciatic gave a strong normal contraction of its muscle group. Soon every reaction upon irritation of the sciatic disappeared. *By the administration of smaller doses of magnesium sulphate and ether the first stage anesthesia was reached and maintained, that is, complete lack of sensation and the necessary general relaxation for all surgical procedures with the maintenance of special reflexes and without any peripheral paralysis.* Meltzer and Auer (1913-14), in collaboration with Peck and Elsberg, employed the combination clinically in seven cases. The patients were given from 0.4-0.6 gram magnesium sulphate per kilo intramuscularly, which was less than half of the effective dose. The ether was administered by endotracheal insufflation. In no case did the patients retain a spark of consciousness even when the concentration of ether in the insufflation was about one-sixth of the concentration usually employed. In all the cases there was complete relaxation of the muscles and in no case was there even the slightest indication of pain while operating in deep, sensitive places, as for example in radical breast amputations or radical removal of extensive, deeply located gland swellings in the neck or laparotomies with manipulation of the parietal peritoneum or gall bladder. With small doses of magnesium sulphate and very lightly maintained ether insufflation, there was often present a certain degree of lid reflex, even at times a spontaneous movement of the lids, without the slightest reaction to the operation on the part of the patient. In such cases the patient awoke shortly after the interruption of the administration of ether and the removal of the insufflation tube, but he could in no way remember the proceedings during the operation.

In order to determine in a quantitative way the extent of the synergism between magnesium sulphate and ether we have applied a technic described by Hooper and Becker, 1924, in which the drugs are injected intravenously into albino rats kept under uniform conditions. We have found that the intravenous administration of less than one-half the anesthetic dose of magnesium sulphate combined with approximately one-ninth the anesthetic dose of ether produces surgical anesthesia without increasing the toxicity of the combination.

EXPERIMENTAL PROCEDURE

Healthy, nonpregnant albino rats weighing approximately 150 grams were employed. The rats were obtained from one source and were kept on a constant well-balanced diet* for at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs when they were weighed and the doses administered per kilo body weight. Water was supplied during the fast period. The solutions of the drugs in distilled water were injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 c.c. The anesthetic dose was considered to have been reached when the application of a pencil point to the eyeball caused no reflex action of the eyelids. The injection was continued at the same rate until the animal stopped breathing when it was assumed the minimal lethal dose had been reached.

TABLE I

ANESTHETIC AND MINIMAL LETHAL DOSES OF ETHER AND MAGNESIUM SULPHATE ALONE OR IN COMBINATION WHEN INJECTED INTRAVENOUSLY INTO ALBINO RATS IN 6 PER CENT AQUEOUS SOLUTIONS

		ANESTHETIC DOSE		MINIMAL LETHAL DOSE	
			Average		Average
		mg. per kg.	mg. per kg.	mg. per kg.	mg. per kg.
Ether	6.0%	646		1985	
NaCl	0.6%	887		2112	
		627	744	1742	1892
		706		1853 (recovered)	
		693		1755	
		905		1905 (recovered)	
MgSO₄	6.0%	180-190 (10 rats)	185	190 (10 rats)	190
Ether	6.0%	180 (90 of each)		316 (158 of each)	
MgSO₄	6.0%	170 (85 of each)	170.8	392 (196 of each)	344.4
		192 (96 of each)		346 (173 of each)	
		140 (70 of each)	(85.4 of each)	344 (172 of each)	(172.2 of each)
		172 (86 of each)		324 (162 of each)	

It will be seen from the table that the anesthetic dose of 6.0 per cent ether is 744 milligrams per kilo body weight and the minimal lethal dose 1892 milligrams. The anesthetic dose is 39.3 per cent of the minimal lethal dose.

The anesthetic dose of 6.0 per cent magnesium sulphate is 185 milligrams per kilo body weight and the minimal lethal dose 190 milligrams. The anesthetic dose is 97.4 per cent of the minimal lethal dose.

The anesthetic dose of a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate is 170.8 milligrams and the minimal lethal dose is 344.4 milligrams. The anesthetic dose is 49.6 per cent of the minimal lethal dose.

Synergism may be defined as the reciprocal augmentation of the action of one drug by that of another. This augmentation is not due to a simple

*Hygienic Laboratory (U. S. P. H. S.) diet. A mixture composed of whole wheat flour, 53.5 per cent; corn meal, 10 per cent; skim milk powder, 33 per cent; calcium carbonate, 0.5 per cent; sodium chloride, 1 per cent; and cod liver oil, 2 per cent.

summation of similar pharmacologic actions because the effect produced is altogether too pronounced.

The table shows that the anesthetic action of ether and magnesium sulphate is definitely synergistic when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate. The anesthetic dose, 170.8 milligrams, contains 85.4 milligrams of ether and magnesium sulphate respectively in combination; 85.4 milligrams ether is 11.5 per cent of the anesthetic dose of straight 6.0 per cent ether (744 milligrams) and 85.4 milligrams magnesium sulphate is 46.2 per cent of the anesthetic dose of straight 6.0 per cent magnesium sulphate (185 milligrams). The total per cent is 57.7 per cent of the combined calculated anesthetic dose. The anesthetic dose of the combination, 170.8 milligrams, represents only 57.7 per cent of the calculated amount required for anesthesia when the drugs are administered singly. In other words there has been a reciprocal potentiation of the anesthetic action by combining magnesium sulphate and ether amounting in this case to approximately 73.5 per cent. We obtained anesthesia with 170.8 milligrams per kilo body weight of the combination. If magnesium sulphate and ether were not synergistic in producing anesthesia, a dose of 296 milligrams would have been required.

The toxicity of ether and magnesium sulphate is apparently additive when injected in combination into the saphenous vein of the albino rat as a water solution containing 6 0 per cent ether and 6.0 per cent magnesium sulphate. The minimal lethal dose 344.4 milligrams contains 172.2 milligrams of ether and magnesium sulphate respectively in combination; 172.2 milligrams ether is 9.1 per cent of the minimal lethal dose of straight 6 per cent ether (1892 milligrams) and 172.2 milligrams magnesium sulphate is 90.6 per cent of the minimal lethal dose of straight 6 per cent magnesium sulphate (190 milligrams). The total per cent is 99.7 per cent of the combined calculated minimal lethal dose. The toxicity of ether and magnesium sulphate in combination is not potentiated. It is a simple summation of the toxicities of the two compounds. The conclusion may therefore be drawn that ether and magnesium sulphate act synergistically in producing anesthesia without producing any increase in toxicity.

II. LOCAL REACTIONS FOLLOWING THE HYPODERMIC INJECTION OF CONCENTRATED MAGNESIUM SULPHATE SOLUTIONS

Meltzer and Auer, 1905, and others state that the subcutaneous injection of concentrated magnesium sulphate solutions into animals may produce abscesses. They further state that in their experiments no aseptic precautions were taken. In our experiments it has been found that abscesses may be avoided by the employment of sterile solutions of chemically pure magnesium sulphate, with asepsis at the time of the injection. We have injected sterile 25 or 50 per cent water solutions subcutaneously or intramuscularly into rats approximately 350 times and have followed over 10,000 hypodermic

injections of the ampuled solution (2 c.c. 50 per cent solution) into human beings without the production of a single abscess or severe tissue reaction.

BACTERIOLOGIC TESTS.—The following bacteriologic tests show that 25 per cent or 50 per cent solutions of magnesium sulphate have little or no germicidal action and that contaminated solutions are not readily sterilized by boiling.

A heavy suspension of an old broth culture of spore-bearing organisms originally obtained from soil was made in ten cubic centimeters of distilled water.

1. Control. One cubic centimeter of the bacterial suspension was added to fifty cubic centimeters of distilled water and thoroughly mixed. The diluted bacterial suspension was placed in resistant glass ampules and sealed. The ampules were then submerged in boiling water 120 minutes (2 hours). Dextrose broth and dextrose agar cultures were then made from the ampuled solution and incubated at 37° C.

Result.—Heavy growth in all tubes within forty-eight hours.

2. One cubic centimeter of the bacterial suspension was added to fifty cubic centimeters of a 25 per cent magnesium sulphate solution in distilled water and thoroughly mixed. The mixture was placed in resistant glass ampules and sealed. The ampules were then submerged in boiling water, five ampules each, for fifteen, thirty and 120 minutes respectively. Dextrose broth and dextrose agar cultures were then made from the ampuled solution and incubated at 37° C.

Result.—Heavy growth in all tubes within forty-eight hours.

3. One cubic centimeter of the bacterial suspension was added to fifty cubic centimeters of a 50 per cent magnesium sulphate solution in distilled water and thoroughly mixed. The mixture was placed in resistant glass ampules and sealed. The ampules were then submerged in boiling water, five ampules each, for fifteen, thirty and 120 minutes respectively. Dextrose broth and dextrose agar cultures were then made from the ampuled solution and incubated at 37° C.

Result.—Heavy growth in all tubes within forty-eight hours.

As shown above, the spore-bearing organisms used in this test withstand boiling temperature (100° C.) under slight presure for at least two hours in the presence of either a 25 per cent or a 50 per cent water solution of magnesium sulphate.

III. THE SIMULTANEOUS ADMINISTRATION OF MAGNESIUM SULPHATE AND MORPHINE

The combination of magnesium sulphate with morphine, in anesthesia, was first suggested by Pellini, who in association with Gwathmey, 1921, determined the effect of this combination by carrying out a sufficient number of dog experiments to prove that one-eighth grain of morphine dissolved in 2 c.c. of a 25 per cent sterile solution of chemically pure magnesium sulphate, given by hypodermic injection and repeated twice at half-hour intervals, analgized the animals sufficiently for a full force of an artery clamp to be placed anywhere on the skin without producing any sign of pain. The control animals given the same amount of morphine in plain water were not analgized to anything like this degree.

After numerous clinical observations, Gwathmey established the fact that morphine may be given in a 50 per cent sterile solution of chemically pure magnesium sulphate instead of plain water and that in this combination the therapeutic value of morphine is increased from 50 to 100 per cent. To illustrate: one-eighth grain of morphine dissolved in 2 c.c. of sterile 50 per cent

magnesium sulphate solution, intramuscularly injected, will relieve pain for from ten to thirty hours, as compared with two to four hours with morphine alone. He states that magnesium sulphate when used as a synergist, exerts no deleterious effect on any of the tissues or organs of the body, including the respiratory center. It seems to act mechanically with morphine, holding it in contact with the tissues longer than the morphine alone is able to maintain such contact. With ether, and with nitrous oxide and oxygen it acts by deepening or increasing the effect, rather than by prolonging it. When magnesium sulphate is used with ether, the latter may be cut one-third to one-half in amount. When magnesium sulphate is used with nitrous oxide and oxygen, the oxygen may be considerably increased and the nitrous oxide decreased.

Gwathmey, 1924, holds that the difficulties experienced with the usual inhalation anesthesia are the lack of relaxation essential for many surgical procedures, and the after-effects of nausea, vomiting, and gas pains, induced by morphine when used alone. By the addition of magnesium sulphate to the preliminary morphine medication, a better relaxation is obtained and the nausea, vomiting, and gas pains are much reduced, if not entirely eliminated. If on the other hand, no allowance is made by the anesthetist, and if a third stage anesthesia is superimposed upon the analgesia induced by preliminary medication, the patient is immediately plunged into the danger zone and possibly into a condition of shock from the inhalation anesthetic. The same degree of relaxation can be obtained with perfect safety by maintaining the first stage of inhalation anesthesia, thus removing the patient from the danger zone by the second and third stages of anesthesia, accomplishing analgesia with unconsciousness. This condition is approximated by using morphine with magnesium sulphate as the analgesic, and inducing unconsciousness with nitrous oxide, ethylene and oxygen.

The hypodermic injection of morphine is occasionally followed by some nausea, which is much more frequently present during recovery from the drug. Evidently the effect of the magnesium sulphate abolishes this delayed untoward effect of morphine. It is well known that the preliminary administration of morphine abolishes the stage of excitement and is a potent factor of safety, since over 90 per cent of all deaths occur in the first five minutes of any inhalation anesthesia. The preliminary injection of morphine renders the anesthesia easier, smoother, and safer. The nausea and vomiting come on later as the effect is wearing off, and are sometimes most disastrous. By using magnesium sulphate with morphine, the morphine effect is retained during the time the patient is emerging from the inhalation anesthesia, and nausea and vomiting are eliminated, as a state of analgesia still exists. Wound and gas pains are likewise reduced to a minimum and quite often are entirely eliminated.

The following case from A. V. S. Lambert's service of the Presbyterian Hospital, New York, illustrates the difference between magnesium sulphate and plain water when used with morphine.

"No. 46241 F.—Badly lacerated wound of leg, gas infection, opened wide December first, gastrocnemius severed.

December		Duration, hours
2nd.	1:15 A.M. Morphine 1/5, MgSO₄ 2 c.c. 25%, quiet until 5...................	5
	6:15 A.M. Morphine 1/12, MgSO₄ 2 c.c. 25%, no pain all day............	18
	The magnesium was now discontinued. Morphine alone used.	
3rd.	1:30 P.M. Morphine 1/6, pain in two hours...........................	2
	3:25 P.M. Morphine 1/6, pain at 6:40 P.M...........................	3¼
	The magnesium was again added to the morphine.	
	6:55 P.M. Morphine 1/6, MgSO₄ 25%, 2 c.c. no pain till next day..........	23
4th.	5:00 P.M. Morphine 1/10, MgSO₄ 2 c.c. 25%, no pain till next A.M..........	17
5th.	10:15 A.M. Morphine 1/10, MgSO₄ 3 c.c. 25%, quiet all day..............	10
	8:15 P.M. MgSO₄ 3 c.c., very good night............................	19
6th.	3:50 P.M. MgSO₄ 3 c.c. 25%, severe pain after 3 hours................:..	3
	6:15 P.M. Morphine 1/8, MgSO₄ 2 c.c. 25%, jerking of leg in 2 hours........	2
	9:15 P.M. Morphine 1/8, MgSO₄ 2 c.c. 25%, quiet night..................	19

The patient required one hypodermic of morphine 1/10 to 1/6 and MgSO₄ 2 c.c. 25 per cent each day after this until December 13. Codeine and morphine alone did not give relief.''

From the above protocol it may be concluded that when the magnesium sulphate (2 to 4 c.c. of a 25 per cent chemically pure solution) is used with morphine, the value of the morphine is increased 50 to 100 per cent. By either increasing or repeating the dose of morphine with sterile water, its action is intensified but not prolonged, as when used with magnesium sulphate.

Smythe, 1923, employed three hypodermic injections of one-eighth grain of morphine dissolved in 2 c.c. of a 25 per cent solution of magnesium sulphate given at twenty minute intervals as a preliminary to novocaine and nitrous oxide-oxygen anesthesia, for general operations. He states that the patient is neither frightened nor apprehensive concerning the operation or its outcome. The stage of induction is greatly shortened and there is rarely a period of excitement. Forty per cent of the patients thus prepared did not require an analgesic after operation. The record of the nurse and bedside notes show that the patients go on an average four times as long after an operation before an analgesic is required as patients who are given morphine dissolved in sterile water alone. The appetite returned earlier in patients operated upon when magnesium sulphate had been employed, because of the absence of pain and restlessness incident to the trauma inflicted at operation.

King, 1923, recommends two or three hypodermic injections of one-eighth grain of morphine dissolved in 2 c.c. of the 25 per cent solution of magnesium sulphate repeated at one-half hour intervals as a preliminary medication for novocaine anesthesia in tonsillectomies. He states that the magnesium sulphate in combination with the morphine produces an analgesic action, so that a small amount of the local anesthetic is very effective.

Weston and Howard, 1923, have injected 2 c.c. or more of a 50 per cent solution of the salt without morphine subcutaneously and intramuscularly more than a thousand times in mental cases with no local pain or sloughing. They state the sedative action occurs in fifteen to thirty minutes and the patient sleeps five to seven hours. In a few instances the patient became quiet but did not sleep. It was found to be a very good substitute for mor-

phine and hyoscine. In 82.7 per cent of the cases it was effective. In 6 per cent the dose was repeated before sedation occurred. In 11 per cent no effect was noted even after three or more doses. They further state the salt is quite harmless in the dose necessary to produce sedative effect and can be given liberally when necessary.

Lederer, 1924, in an article entitled synergistic analgesia as employed in the first 1000 cases about the head and neck, advocates the use of three intramuscular injections of one-eighth grain of morphine dissolved in 2 c.c. of a 25 per cent solution of magnesium sulphate with a rectal instillation containing equal parts of olive oil and ether (each 3 ounces) and 2 drams of paraldehyde. The hypodermic injections were given at thirty minute intervals beginning three to four hours prior to operation. The rectal instillation was begun thirty minutes after the last hypodermic injection. Supplementary anesthesia, either by local infiltration or by drop ether was required in 12 per cent of the cases. There was no excitement stage present during the induction of the analgesia. Time in regard to the duration of the operation was a negligible factor, as the narcosis was smooth and of uniform depth for hours. At no time was there cyanosis or loss of body heat. Recovery from the analgesia was rapid; was not followed by lack of appetite; nor was there any tendency to mental depression. Postoperative medication was required in less than 0.5 per cent of the cases and then only in small amounts. Signs of irritation of the gastrointestinal tract or respiratory tract were never present. There were no kidney or bladder disturbances. One patient received the analgesic fourteen times in the course of three years without noticing any untoward symptoms or drug tolerance.

Gwathmey and his collaborators, 1923 and 1924, are perfecting a synergistic method for inducing obstetric analgesia. The procedure is based in great part upon the synergistic action of magnesium sulphate and ether. The technic, developed after experience with over 1000 cases, calls for three hypodermic injections and one rectal instillation. The first hypodermic injection is given when the pains are four or five minutes apart and lasting thirty or more seconds. It consists of one-sixth grain of morphine, dissolved in 2 c.c. of a 50 per cent solution of magnesium sulphate. The morphine is not repeated. The rectal instillation which follows within twenty minutes, if no relief is afforded by the first hypodermic injection, contains quinine hydrobromide, 20 grains; alcohol, 3 drams; ether, 2½ ounces, and olive oil, q. s. ad 4 ounces. If the first hypodermic injection produces a marked sedative effect, the instillation is delayed from one to two hours. The effect of the hypodermic and instillation continues for about four hours, but if insufficient, one or two additional hypodermic injections of 2 c.c. 50 per cent magnesium sulphate solution are given to deepen the effect. The instillation may also be repeated after four hours. Pain is eliminated in over 90 per cent of the cases, labor is not delayed, and memory of events is either clouded or completely obliterated. The condition of the baby is not affected by the medication. It is the exception to have delayed respiration.

Adams, 1924, employed one or two intramuscular injections of one-fourth

grain morphine dissolved in 2 c.c. of a 25 per cent solution of magnesium sulphate as an obstetric analgesia in sixty cases. The initial injections were made into the deltoid muscle when the cervix was on the average two and one-half centimeters dilated. In thirteen cases where the effect of the medication seemed favorable but not lasting, the injections were repeated. The average dilatation at the time of the second injection was four and one-half centimeters. There was a definite sedative effect on the labor pains in the large majority of cases (93 per cent) without increasing the fetal or maternal morbidity or mortality. The total length of labor was not prolonged. Postpartum bleeding was not excessive. The uterus at all times retained a good tone and no abnormal tendency to relaxation was noted. The author concludes that the treatment possesses possibilities as a simple, inexpensive and apparently harmless method of obstetric analgesia.

IV. ANALGESIC, ANESTHETIC AND TOXICITY EXPERIMENTS WITH MAGNESIUM
SULPHATE ALONE; MAGNESIUM SULPHATE AND MORPHINE; MAGNESIUM
SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE,
MORPHINE AND NOVOCAINE

In order to make our experiments of practical value, and for comparative purposes, the following sterile solutions were employed in the subcutaneous experiments:

1. Magnesium sulphate 50 per cent.
2. Magnesium sulphate 50 per cent with morphine sulphate 0.4 per cent (2 c.c. contains one-eighth grain morphine).
3. Magnesium sulphate 50 per cent with novocaine 2.5 per cent.*
4. Magnesium sulphate 50 per cent with morphine 0.4 per cent and novocaine 2.5 per cent.

In the intravenous experiments the above solutions were diluted with a sufficient quantity of distilled water to make a 6 per cent magnesium sulphate content.

In the computation of the amount of solution to be injected at a given dose the magnesium sulphate content of the solution alone was considered. The amount of novocaine and morphine contained in the solutions was disregarded.

SUBCUTANEOUS EXPERIMENTS WITH ALBINO RATS

Healthy, nonpregnant albino rats weighing between 100 and 150 grams were employed. The rats were obtained from one source and were kept on the Hygienic Laboratory (U.S.P.H.S.) diet at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs when they were weighed and the doses were administered per kilo body weight. Water was supplied during the fast period. The hair over the back above the tail was clipped and the injections were

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*In the preliminary subcutaneous experiments in which a 50 per cent water solution of magnesium sulphate was used alone and in combination with 1.25 per cent, 2.5 per cent, 5 per cent, and 10 per cent novocaine, it was found that the most suitable combination for the production of analgesia and anesthesia was a 50 per cent magnesium sulphate solution with 2.5 novocaine. The latter combination was therefore adopted for the more detailed experiments.

made subcutaneously in the left lumbar region. The animals were kept under observation until they completely recovered. The analgesic dose was considered to have been reached when the corneal and tail reflexes were absent or markedly diminished with the animal otherwise normal or somewhat de-

TABLE II

COMPARISON OF THE ANALGESIC AND ANESTHETIC DOSES OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND MORPHINE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, MORPHINE AND NOVOCAINE. ALBINO RATS INJECTED SUBCUTANEOUSLY

		DOSE	RESULTS OBTAINED
		mg. per kg.	
$MgSO_4$	50.0%	400	Active and normal. Corneal and tail reflexes maintained
		800	Active and normal. Corneal and tail reflexes maintained
		1000	Depression within 20 minutes lasting approximately 25 minutes. Corneal and tail reflexes maintained. Does not assume dorsal position
		1000	Some depression within 27 minutes lasting approximately 19 minutes. Corneal reflex somewhat diminished. Tail reflex maintained. Does not assume dorsal position
		1200	*Complete analgesia within 20 minutes. Corneal and tail reflexes absent 12 minutes. Does not assume dorsal position*
		1400	*Complete anesthesia with dorsal position, relaxation and absence of corneal and tail reflexes within 14 minutes lasting approximately 28 minutes*
$MgSO_4$	50.0%	400	Active and normal. Corneal and tail reflexes maintained
Morphine		600	Active and normal. Corneal and tail reflexes maintained
Sulphate	0.4%	800	*Definite analgesia within 23 minutes. Corneal and tail reflexes absent approximately 15 minutes. Does not maintain dorsal position*
		1000	*Complete analgesia within 15 minutes and complete anesthesia within 21 minutes. Corneal and tail reflexes absent approximately 70 minutes. Maintained dorsal position approximately 30 minutes*
$MgSO_4$	50.0%	600	Active and normal. Corneal and tail reflexes maintained
Novocaine	2.5%	800	*Definite analgesia within 19 minutes. Corneal and tail reflexes absent or markedly diminished for approximately 30 minutes. Does not maintain dorsal position*
		1000	*Complete anesthesia, with dorsal position, relaxation, automatic breathing and absence of corneal and tail reflexes within 20 minutes lasting approximately 30 minutes. Complete recovery within 1 hour*
$MgSO_4$	50.0%	200	Active and normal. Corneal and tail reflexes maintained
Novocaine	2.5%	400	Definite analgesia within 32 minutes. Corneal and tail reflexes markedly diminished approximately 30 minutes
Morphine		600	*Complete analgesia within 25 minutes. Corneal and tail reflexes absent approximately 40 minutes*
Sulphate	0.4%	800	*Complete analgesia within 24 minutes and complete anesthesia within 30 minutes. Corneal and tail reflexes absent approximately 72 minutes. Remained in dorsal position 25 minutes*
(1/8 grain of morphine in 2 c.c. solution)		1000	Complete analgesia within 20 minutes and complete anesthesia within 32 minutes. Corneal and tail reflexes absent approximately 130 minutes. Maintained dorsal position 40 minutes

pressed. The anesthetic dose was considered to have been reached when the animal maintained a dorsal position with relaxation, automatic respiration and absence of corneal and tail reflexes. The corneal reflex was considered absent when the application of a pencil point to the cornea caused no reflex action of the eyelids. The tail reflex was obtained by applying a mouse

tooth forceps to the base of the tail with sufficient force to prick the skin. A normal animal will invariably swing around and bite the forceps.

Animal experiments dealing with drug combinations are difficult, usually unsatisfactory, and in order to draw accurate conclusions a large number of well-controlled experiments are required. In Table II, eighteen representative experiments are tabulated. Approximately two hundred and seventy-five experiments of this type have been carried out.

In the first column are given the solutions employed; in the second column the dose in milligrams per kilo body weight; in the third column. a summary of the effects produced.

Table II shows the analgesic dose of magnesium sulphate for the albino rat, when injected in 50 per cent water solution, is approximately 1200 milligrams per kilo and the anesthetic dose 1400 milligrams.

The analgesic dose of 50 per cent magnesium sulphate with 0.4 per cent morphine sulphate is 800 milligrams and the anesthetic dose 1000 milligrams per kilo.

The analgesic dose of 50 per cent magnesium sulphate with 2.5 per cent novocaine is also 800 milligrams and the anesthetic dose 1000 milligrams.

The analgesic dose of 50 per cent magnesium sulphate solution with 0.4 per cent morphine and 2.5 per cent novocaine is between 400 and 600 milligrams and the anesthetic dose 800 milligrams. The addition of 2.5 per cent novocaine to the magnesium sulphate solution with morphine (one-eighth grain morphine in 2 c.c. solution) increases the analgesic and anesthetic actions of the combination at least 25 per cent and also definitely prolongs the effect.

TABLE III

COMPARISON OF THE TOXICITY OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, MORPHINE AND NOVOCAINE. RATS INJECTED SUBCUTANEOUSLY. PERCENTAGE OF RATS THAT DIED

		DOSE EXPRESSED IN MILLIGRAMS PER KILO BODY WEIGHT							
		1100	1200	1300	1400	1500	1600	1800	2000
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
MgSO₄	50.0%					0(5)	20(5)	20(5)	20(5)
MgSO₄ Novocaine	50.0% 2.5%		0(5)	0(5)	20(5)	60(5)			
MgSO₄ Novocaine Morphine	50.0% 2.5% 0.4%	0(5)	80(5)	100(5)	100(5)	100(5)			

The numbers within the parentheses represent the number of rats used in the series.

The technic of the experiments presented in Table III was the same as that used for the experiments tabulated in Table II except that a series of rats was run at each dose, regardless of analgesia or anesthesia. The animals were kept under observation for twenty-four hours, during which time they were fed the routine diet.

It will be seen from Table III that the maximal tolerated dose (minimal dose at which any of a series of animals dies) is 1600 milligrams per kilo for the 50 per cent magnesium sulphate solution; 1400 milligrams for the 50

per cent magnesium sulphate solution with 2.5 per cent novocaine; and 1200 milligrams for the 50 per cent magnesium sulphate solution containing 2.5 per cent novocaine and 0.4 per cent morphine. These results are based upon subcutaneous injections. They are in marked contrast with the results later presented in this article, where the solutions were injected intravenously and the toxicity found to be approximately the same. The discrepancy between

TABLE IV

COMPARISON OF THE ANALGESIC DOSE OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND MORPHINE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, NOVO-CAINE AND MORPHINE. RATS INJECTED INTRAVENOUSLY

		ANALGESIC DOSE		DURATION OF ANALGESIA	REMARKS
			AVERAGE		
MgSO$_4$	6.0%	mg. per kg. 180-190	mg. per kg. 185 (15 rats)	minutes 1-2	
MgSO$_4$ Morphine Sulphate	6.0% 0.048%	43 64 40 39 34 32 56 33 44 44	42.9 (10 rats).	10-45	Solution in proportion to that used in the subcutaneous experiments
MgSO$_4$ Novocaine	6.0% 0.3%	65 57 67 70 44 43 32 57 53 77 43 59 65 43 43	54.5 (15 rats)	2-5	Solution in proportion to that used in the subcutaneous experiments
MgSO$_4$ Novocaine Morphine Sulphate	6.0% 0.3% 0.048%	40 36 24 30 40 33 27 40 30 34 34 29 31 32 22 26 42 36 30 33	32.4 (20 rats)	15-60	Solution in proportion to that used in the subcutaneous experiments

the subcutaneous and intravenous experiments may possibly be due to difference in the rate of absorption of the solutions from the subcutaneous tissues. In our opinion the intravenous experiments are much more reliable.

INTRAVENOUS EXPERIMENTS WITH ALBINO RATS

In the following experiments the technic was the same as in the previous experiments except that the solutions of the drugs in distilled water were injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 c.c. The analgesic dose was considered to have been reached when the application of a pencil point to the cornea caused no reflex action of the eyelid.

In carrying out the toxicity experiments, five rats were injected at each dose regardless of analgesia or anesthesia. The animals were kept under observation for twenty-four hours after injecting the solutions and were fed the routine diet.

Table IV shows that the analgesic dose of 6 per cent magnesium sulphate solution injected into the saphenous vein of the albino rat is approximately 185 milligrams per kilo. The analgesic dose of 6 per cent magnesium sulphate solution with 0.048 per cent morphine is 42.9 milligrams. The analgesic dose of 6 per cent magnesium sulphate solution with 0.3 per cent novocaine is 54.5 milligrams. The analgesic dose of 6 per cent magnesium sulphate solution with 0.3 per cent novocaine and 0.048 per cent morphine is 32.4 per cent. Further, the addition of the morphine prolongs the effect.

TABLE V

A QUANTITATIVE COMPARISON OF THE TOXICITY OF MAGNESIUM SULPHATE ALONE; MAGNESIUM SULPHATE AND NOVOCAINE; AND MAGNESIUM SULPHATE, NOVOCAINE AND MORPHINE. RATS INJECTED INTRAVENOUSLY. PERCENTAGE OF RATS THAT DIED

		DOSE EXPRESSED IN MILLIGRAMS PER KILO BODY WEIGHT		
		180	190	200
		per cent	per cent	per cent
$MgSO_4$	6.0%	0(5)	100(5)	80(5)
$MgSO_4$ Novocaine	6.0% 0.3%	0(5)	20(5)	20(5)
$MgSO_4$ Novocaine Morphine sulphate	6.0% 0.3% 0.048%	0(5)	60(5)	80(5)

The numbers within the parentheses represent the number of rats used in the series.

It will be seen from Table V that the addition of 0.3 per cent novocaine to a 6 per cent water solution of magnesium sulphate or the addition of 0.3 per cent novocaine and 0.048 per cent morphine sulphate, does not increase the toxicity of a 6 per cent magnesium sulphate solution when injected into the saphenous vein of the albino rat at the rate of twelve to fifteen seconds for each 0.1 c.c.

EXPERIMENTS WITH DOGS AND CATS

Beckman of Marquette University School of Medicine (personal communication)* has found that the subcutaneous injection of 2 c.c. of 50 per

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cent magnesium sulphate solution simultaneously with 0.25 c.c. of a 4 per cent solution of morphine per kilo into dogs is followed by certain undesirable symptoms which he attributes to the magnesium sulphate. He states: "These dogs have been much more depressed when brought into the anesthetizing room than those given morphine alone; they are whining as though in distress; the rate of respirations is not changed but the expirations are jerky (there is almost an expiratory grunt); and in most cases there has been a quite profuse salivation." The dose of morphine employed, 0.25 c.c. of a 4 per cent solution per kilo, represents a dose of 10 milligrams (one-sixth grain) per kilo or 110 milligrams (one and three-fourth grains) for a dog weighing 11 kilos (24.2 lbs.). The dose of morphine is very large and out of all proportion to the amount of magnesium sulphate used. Beckman's experiments show definitely that the combined administration of magnesium sulphate and morphine in disproportionate amounts is not practicable and that it would no doubt prove decidedly dangerous in clinical application. The combination of magnesium sulphate and morphine as employed in our experiments (2 c.c. 50 per cent magnesium sulphate solution with one-eighth grain of morphine) is administered clinically almost constantly with no untoward results. Several thousand of these injections are now being given every month. Based on animal experiments a clinical dose of 2 c.c. 50 per cent magnesium sulphate solution administered subcutaneously or intramuscularly is at least 100 times removed from the fatal dose. Meltzer and Auer, 1905, demonstrated conclusively that dogs withstand by subcutaneous injection 1.5 grams magnesium sulphate per kilo without showing any distress or respiratory symptoms. The dose of 1.5 grams magnesium sulphate per kilo represents a dose of 33 c.c. of a 50 per cent magnesium sulphate solution for a dog weighing 11 kilos (24.2 lbs.) or 225 c.c. for a man weighing 75 kilos (165 lbs.). They further found that the subcutaneous injection of 2 grams magnesium sulphate per kilo produced a profound anesthesia and general paralysis which led sooner or later to a calm death without being preceded or accompanied by any symptoms of excitation.

We have administered eight intramuscular injections at twenty minute intervals of either 2 c.c. 50 per cent magnesium sulphate solution with 0.4 per cent morphine (one-eighth grain in 2 c.c.), or 2 c.c. 50 per cent magnesium sulphate solution with 0.4 per cent morphine and 2.5 per cent novocaine into dogs weighing between eleven and twelve kilos. Each animal received 8 grams magnesium sulphate (661 milligrams per kilo, average) with 1 grain of morphine. They showed no untoward symptoms and the respirations remained normal throughout the experiments. There was some salivation but no vomiting or defecation following the injections of magnesium sulphate with morphine alone. There was no salivation, vomiting or defecation following the injections of magnesium sulphate, morphine and novocaine. The animals became definitely analgized, remained calm and quiet for several hours and were completely recovered by the following morning. In other experiments we administered single intramuscular injections of 8 c.c. 50 per cent magnesium sulphate solution containing one-half grain of morphine

both with and without the novocaine into dogs weighing seven to twelve kilos.   None of the animals showed any untoward symptoms.   A striking feature has been the absence of the defecation and vomiting which usually follows the injection of simple morphine solution.

The subcutaneous or intramuscular injections of a fatal or nearly fatal dose of morphine in the cat (20 milligrams per kilo) has an exciting effect. The animal races about as if in extreme terror, with prominent eyes and widely dilated pupils scratching and clawing wildly.   Sometimes there are even convulsions.   In all of the experiments that Meltzer and his collaborators carried out with the injection of magnesium sulphate, not a single instance was observed in which this salt produced an increase of excitation; on the contrary any effect which it produced was invariably in the direction of a reduction of excitation or of its complete temporary or permanent abolition.   With these facts in mind eight cats were injected intramuscularly, three with morphine alone (20 milligrams per kilo) and five with the morphine dissolved in 50 per cent magnesium sulphate solution with 2.5 per cent novocaine (800-1200 milligrams $MgSO_4$ per kilo).   The three control cats developed a violent mania.   The animals that received the morphine dissolved in magnesium sulphate and novocaine did not become violent and four of the animals soon became deeply anesthetized.   The experiments show that magnesium sulphate is capable of reducing or completely inhibiting the symptoms of excitation which follow the injection of morphine alone in the cat.

SUMMARY

The synergistic action of magnesium sulphate and ether is described on a quantitative basis.

Magnesium sulphate and ether act synergistically in producing anesthesia when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate.   The synergism is of the potentiated type.

The toxicity of magnesium sulphate and ether is additive when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent ether and 6.0 per cent magnesium sulphate.   The toxicity is a simple summation of the toxicities of the two compounds.

The anesthetic dose of magnesium sulphate approaches the fatal dose very closely when injected into the saphenous vein of the albino rat as a 6.0 per cent water solution or when injected subcutaneously as a 50 per cent water solution.   The margin of safety is slight.

The anesthetic dose of ether represents approximately 40 per cent of the minimal lethal dose when injected into the saphenous vein of the albino rat as a 6.0 per cent water solution.

The anesthetic dose of magnesium sulphate and ether represents approximately 50 per cent of the minimal lethal dose when injected in combination into the saphenous vein of the albino rat as a water solution containing 6.0 per cent magnesium sulphate and 6.0 per cent ether.

Concentrated magnesium sulphate solutions have little or no germicidal action. Contaminated solutions are not readily sterilized by boiling.

Sterile 25 or 50 per cent water solutions of specially tested, chemically pure magnesium sulphate do not produce abscesses or severe tissue reactions when injected with asepsis subcutaneously or intramuscularly into the albino rat or the human being (2 c.c. injected at one site).

A review of the literature dealing with the simultaneous administration of magnesium sulphate and morphine is given.

Comparative analgesic, anesthetic, and toxicity experiments with magnesium sulphate alone; magnesium sulphate and morphine; magnesium sulphate and novocaine; and magnesium sulphate, morphine and novocaine are given in which the drugs were injected subcutaneously and intravenously into the albino rat.

The addition of 2.5 per cent novocaine to a 50 per cent magnesium sulphate solution with morphine sulphate 0.4 per cent (2 c.c. contains one-eighth grain morphine) increases the analgesic and anesthetic actions of the combination at least 25 per cent and also definitely prolongs the effect when injected subcutaneously or intravenously into the albino rat.

In a limited number of experiments with dogs the subcutaneous or intramuscular administration of morphine dissolved in 50 per cent magnesium sulphate solution with 2.5 per cent novocaine (one-eighth grain morphine in 2 c.c.) has not been followed by salivation, vomiting or defecation.

The simultaneous administration of magnesium sulphate 50 per cent solution with 2.5 per cent novocaine (800-1200 milligrams magnesium sulphate per kilo) reduces or completely inhibits the symptoms of excitation which follow the subcutaneous injection of a fatal or nearly fatal dose of morphine in the cat.

Based on animal experiments the clinical dose of 2 c.c. 50 per cent magnesium sulphate solution administered subcutaneously or intramuscularly is at least 100 times removed from the fatal dose.

Magnesium sulphate used alone in sufficient amount to produce anesthesia is the most dangerous anesthetic known. When used as a synergist with other drugs it may become one of the most valuable drugs in the pharmacopeia.

REFERENCES

Adams, T. W.: Morphine and Magnesium Sulphate as an Obstetrical Analgesic. Am. Jour. Obst. and Gynec., 1924, viii, 266-271.

Auer, J., and Meltzer, S. J.: The Intravenous Injection of Magnesium Sulphate for Anesthesia in Animals. Jour. Exper. Med., 1916, xxiii, 641-653.

Gates, F. L., and Meltzer, S. J.: An Experimental Study of the Additive and Antagonistic Actions of Sodium Oxalate, and Salts of Magnesium and Calcium in the Rabbit. Jour. Exper. Med., 1916, xxiii, 655-667.

Gwathmey, J. T.: Synergistic Colonic Analgesia. Jour. Am. Med. Assn., 1921, lxxvi, 222-225.

Gwathmey, J. T.: Anesthesia. N. Y. and London, 1924, Macmillan Company, 799 pp., 8°.

Gwathmey, J. T., Donovan, E. P., O'Reagan, J., and Cowan, L. R.: Painless Childbirth by Synergistic Methods. Am. Jour. Obst. and Gynec., 1923, vi, 456-466.

Gwathmey, J. T., and Greenough, J.: Synergistic Analgesia. Am. Jour. Surg., Anesthesia Supplement, 1922, xxxvi, 110-114.

Gwathmey, J. T., and Greenough, J.: Experiences with Synergistic Anesthesia. Am. Jour. Surg., Anesthesia Supplement, 1922, xxxvi, 22-25.

Gwathmey, J. T., McKenzie, R. A., and Hudson, F. J.: Painless Childbirth by Synergistic Methods (Second Paper). Am. Jour. Obst. and Gynec., 1924, viii, 154-163.

Haubold, H. A., and Meltzer, S. J.: Spinal Anesthesia by Magnesium Sulphate. Jour. Am. Med. Assn., 1906, xlvi, 647-650.

Henderson, V. E.: Anesthesia by the Intracerebral Injection of Magnesium Chloride. Jour. Pharm. and Exper. Ther., 1909, i, 199-201.

Hooper, C. W., and Becker, E.: A Quantitative Comparison of the Toxicity of the Alkamine Esters of Aromatic Acids Used as Local Anesthetics. JOUR. LAB. AND CLIN. MED., 1924, x, 43-46.

Joseph, D. R., and Meltzer, S. J.: The Comparative Toxicity of the Chlorides of Magnesium, Calcium, Potassium and Sodium. Jour. Pharm. and Exper. Ther., 1909, i, 1-26.

Joseph, D. R., and Meltzer, S. J.: The Life-Saving Action of Physostigmin in Poisoning by Magnesium Salts. Jour. Pharm. and Exper. Ther., 1909, i, 369-387.

King, J. J.: Tonsillectomy in Adults under Local Anesthesia. Internat. Jour. Surg., 1923, xxxvi, 141-146.

Lederer, F. L.: Synergistic Analgesia as Employed in the First 1000 Cases about the Head and Neck. Am. Jour. Surg., Anesthesia Supplement., 1924, xxxviii, 80-88.

Mansfeld, G.: Ueber das Wesen synergetische Arzneiwirkung. I. Mitteilung. Arch. f. d. ges. Physiol., 1915, clxi, 444-460.

Mansfeld, G.: Experimentelle Untersuchungen über Wesen und Aussicht der Tetanustherapie mit Magnesiumsulfat. München. med. Wchnschr., 1915, lxii, 208.

Mansfeld, G., and Bosanyi, S.: Untersuchungen über das Wesen der Magnesiumnarkose. Arch. f. d. ges. Physiol., 1913, clii, 75-80.

Meltzer, S. J.: Inhibitory and Anesthetic Properties of Magnesium Salts. Medical Record, 1905, xlviii, 965.

Meltzer, S. J.: The Influence of Diuresis upon the Toxic Dose of Magnesium Salts. The Toxicity of Magnesium Nitrate When Given by Mouth. Proc. Soc. Exper. Biol. and Med., 1907, iv, 140-143.

Meltzer, S. J.: The Relation of the Purgative Action of Magnesium Sulphate to Peristalsis, and the General Law of Crossed Innervation. Arch. Int. Med., 1905, xv, 955-963.

Meltzer, S. J., and Auer, J.: Physiological and Pharmacological Studies of Magnesium Salts. I. General Anesthesia by Subcutaneous Injections. Am. Jour. Physiol., 1905, xiv, 366-388.

Meltzer, S. J., and Auer, J.: Physiological and Pharmacological Studies of Magnesium Salts. II. The Toxicity of Intravenous Injections; in Particular the Effects upon the Centres of the Medulla Oblongata. Am. Jour. Physiol., 1906, xv, 387-405.

Meltzer, S. J., and Auer, J.: Physiological and Pharmacological Studies of Magnesium Salts. III. The Narcotizing Effect of Magnesium Salts upon Nerve Fibres. Am. Jour. Physiol., 1906, xvi, 233-251.

Meltzer, S. J., and Auer, J.: Physiological and Pharmacological Studies of Magnesium Salts. IV. The Relations of the Salts to the Peristalsis of the Gastro-Intestinal Canal. Am. Jour. Physiol., 1906, xvii, 313-320.

Meltzer, S. J., and Auer, J.: The Antagonistic Action of Calcium upon the Inhibitory Effect of Magnesium. Am. Jour. Physiol., 1908, xxi, 400-419.

Meltzer, S. J., and Auer, J.: The Action of Strontium Compared with that of Calcium and Magnesium. Am. Jour. Physiol., 1908, xxi, 449-453.

Meltzer, S. J., and Auer, J.: Is the Anesthesia and Motor Paralysis Caused by Magnesium Salts Due to Asphyxia? Am. Jour. Physiol., 1908, xxiii, 141-147.

Meltzer, S. J., and Auer, J.: Combined Action of Magnesium and Ether, Evidence of a Central Effect of Magnesium. Proc. Soc. Exper., Biol. and Med., 1912-13, x, 159-161.

Meltzer, S. J., and Auer, J.: Ueber die anästhetische und lähmende Wirkung von Magnesium unterstützt durch Aether. Zentralbl. f. Physiol., 1913-14, xxvii, 632-635.

Meltzer, S. J., and Lucas, D. R.: Physiological and Pharmacological Studies of Magnesium Salts. V. The Influence of Nephrectomy upon Their Toxicity. Jour. Exper. Med., 1907, ix, 298-311.

Peck, C. H., and Meltzer, S. J.: Anesthesia in Human Beings by Intravenous Injection of Magnesium Sulphate. Jour. Am. Med. Assn., 1916, lxvii, 1131-1133.

Rector, J. M.: Synergistic Analgesia: Clinical Observations. Am. Jour. Surg., Anesthesia Supplement, 1922, xxxvi, 114-119.

Smythe, F. D.: Clinical Experience with Synergistic Analgesia in a Series of Major Operations. Memphis Med. Month, 1921, xlii, 237.

Smythe, F. D.: Synergistic Analgesia. Am. Jour. Surg., Anesthesia Supplement, 1923, xxxvii, 85-89.

Straub, W.: Experimentelle Untersuchung über Wesen und Aussicht der Tetanustherapie mit Magnesiumsulfat. München. med. Wchnschr., 1915, lxii, 25-27.

von Issekutz, B.: Ueber die kombinierte Wirkung des Magnesiumsulfats mit verschiedener Narkotiker. Therap. Monatsch., 1915, xxix, 379-384.

Weston, P. G., and Howard, M. Q.: Magnesium Sulphate as a Sedative. Am. Jour. Med., Sc., 1923, clxv, 431.

ACUTE MYELOID LEUCEMIA*

(Report of a case)

By A. A. Blair, M.D., Fort Smith, Arkansas

THE incidence of acute myeloid leucemia is exceedingly rare. In fact we are skeptical in accepting our findings, often in making a diagnosis, owing to the few cases reported and variability of blood findings. However, during the past few years sufficient clinical and microscopic data has been obtained to clear up this doubt. Clinically it is characterized by sudden onset, short and stormy course, hyperpyrexia, rapid and progressive anemia, usually infective and ulcerative changes of the oral cavity with frequent hemorrhages from the mucous membranes, terminating in death in from four weeks to four months.

The suggestion that it is of infectious origin is well understood, being frequently associated with dental infection, ulcerative tonsillitis, etc. According to Gorham† to make a diagnosis of the acute form of this disease, the patient should present specific findings, beginning with a subleucemic stage, later development of a characteristic blood picture of myeloblasts and myelocytes and certain transition forms (proving the myeloid elements by the oxidase reaction), with typical gross and histologic findings and early death. Without going into discussion of all the phases of the disease, I wish to present a case, certainly one of the above type.

Patient, M. A., consulted us first July 14, 1924. White female, age fifty-three (married thirty-four years), three children living, one miscarriage. Previous history of no importance. Clinical history shows that she had influenza in February this year, but has been in fair health until present attack. Three weeks prior to July 14, she had six teeth extracted by her dentist. Since this time her gums have been badly infected and she has had pain in her face and neck, with elevation of temperature, pain in her arms and legs and progressive weakness, menorrhagia and metrorrhagia. Her first examination of July 14, 1924, revealed the following briefly:

A small sallow individual 51 inches in height, 91 pounds. Blood pressure 120/64; temperature 100° F.; cheeks pale; hair on head and pubis scanty; mouth showed marked bad dental sepsis, gums swollen and bleeding occasionally, enlargement of cervical glands; skin over chest and part of abdomen shows numerous small intracutaneous nodules about the size of a pea. Heart examination negative. Chest showed a few moist râles in left apex, not persistent; expansion good. Abdomen: Spleen palpable. Vaginal examination: Uterus slightly

enlarged, and soft, old cervical laceration present. Reflexes were normal. Laboratory examination: Urine showed a heavy cloud of albumin; many granular casts, red and pus cells; sputum negative; stool negative; blood Wassermann negative; blood culture negative; malaria negative; hemoglobin 39 per cent (Sahli). R. B. C. 2,000,000; total white count 24,400; differential count, polymorphonuclear neutrophile 23 per cent; small lymphocytes 10 per cent; large lymphocytes 2 per cent; eosinophiles 2 per cent; neutrophilic myelocytes 35 per cent; myeloblasts 25 per cent; smears obtained from ulcerations of gums showed numerous fusiform bacilli and spirilla. Patient was ordered to Sparks Memorial Hospital for observation and treatment. Arsenic in the form of sodium cacodylate and Fowler's solution was pushed. Cleansing solution for mouth was given.

Progress, July 16, 1924, patient appeared some better. Temperature 100° F. axillary; not so much pain in face; blood culture negative.

July 17. Temperature 100.2° F. Condition about the same. Spleen on level of umbilicus.

July 18 to 24. No noticeable improvement during this time. X-ray treatment over spleen and long bones given every third day.

July 25. Temperature rose to 103° F.; pulse 120. Patient felt very uncomfortable. Sense of choking in throat, bleeding from the gums, pain in face; ¼ gr. morphine given. Blood culture negative; stool examination positive for blood.

July 26. Temperature 104° F. Spleen could be easily felt by dipping fingers over crest of ileum, lower border three finger width below umbilicus in median line, rapidly filling left side of abdomen. Some apparent enlargement of the liver; tender over shafts of long bones; white blood count 35,000; red blood count 1,800,000; hemoglobin 33 per cent; polymorphonuclear neutrophile 22 per cent; small lymphocytes 11 per cent; large lymphocytes 1 per cent; myelocytes, neutrophilic 36 per cent; myeloblasts 31 per cent. Predominating myelocytes and myeloblasts being from 20 to 23 microns, the oxidase reaction not performed.

The temperature between July 26 and Aug. 3 ranged from 102° F. to 104° F.; pulse 120 to 150. Patient nauseated and vomiting. Pain in face and limbs only relieved by morphine; very nervous and tossing about in bed; rational only part of time.

Died at 5 P.M. Aug. 4, 1924. Permission for autopsy on this patient was not given.

LABORATORY METHODS

A SIMPLE THERAPEUTIC METHOD FOR THE STANDARDIZATION OF DIGITALIS*

By J. B. Berardi, Ph.G., B.S., M. C. Canan, B.S., and H. McGuigan, M.D.

THE available methods for the standardization of digitalis are far from satisfactory. The frog method which is most used requires several animals and a considerable amount of time to carry out the standardization. The results are frequently inconsistent and unsatisfactory. The cat method also often gives variable results, and as the standardization is done under an anesthetic, it is far removed from the condition in which digitalis is employed in the practice of medicine. The present investigation is an attempt to approximate therapeutic usage and at the same time devise a simple and available method. This procedure consists briefly in the intravenous injection of the drug into a normal dog without anesthesia. The heart rate is counted before and after the drug and the slowing of the rate is taken as a measure of the activity of the drug. If the preparation be of standard strength, 0.02 c.c. of the tincture per kilo weight of dog, will reduce his heart rate 20 per cent in from thirty to sixty minutes. The animal is not injured and may be used again for the same purpose in the course of a few days. The details of the method are as follows:

A dog is weighed. The normal heart rate is then determined by taking an average of four or five results at ten minute intervals. It is important to get an accurate count without excitement. A definite amount of tincture of digitalis diluted with physiologic salt solution is then given intravenously. After the injection the animal is carefully watched, the heart rate being taken every ten minutes until the greatest drop has been recorded and the heart shows signs of returning to normal. From this data the percentage drop is calculated. Observations upon respiration showed no marked variations, hence respiration is not considered in the standardization.

The first series of dogs were injected with ¼ c.c. of tincture diluted in salt solution. This was a dosage of from 0 017 to 0.055 c c. per kilogram. We found, in seven dogs examined, that there was no uniform time before a change in the heart rate was observed, but that the percentage slowing showed a definite relation to the dosage per kilogram body weight and that the greatest slowing occurred between thirty and sixty minutes after injection. Some of these animals were used several times in order to determine the accumulative effect of the drug.

DIGITALIS

	WEIGHT IN KILOS	DOSE IN C.C.	DOSE PER KILO	NORMAL HEART RATE	DIGITALIS HEART RATE	TIME FOR DROP	AMOUNT OF DROP IN BEATS	PER CENT SLOWING OF HEART	
TABLE I	14.09	¼	0.017	107.5	68	30'	39.5	36	Male
	13.3	¼	0.018	98.5	68	30'	30.5	30	Female
	6.8	¼	0.036	124.5	76	19'	48.5	39	Male
	8.7	¼	0.028	108.5	88	5'	20.5	19	Female
	4.5	¼	0.055	178	110	10'	68	38	Female
	12.5	¼	0.020	117.5	80	17'	37.5	31	Male
	11	¼	0.022	104.7	88	2'	14.7	14	Male
TABLE II	12	½	0.041	137.5	120	5'	17.5	12.5	Female
	5.6	½	0.088	142	120	5'	22.1	15	Male
	5	½	0.10	130	72	30'	58	44	Female
TABLE III	20	1	0.05	82	59	1 hr. 44'	23	27	Male
	20	1	0.05	104	80	1 " 21'	34	32	Male
	18.1	1	0.055	110	90	1 " 33'	30	27	Male
	11.5	1	0.086	109	76	33'	33	30.2	Male
TABLE IV	59	2	0.35	114	90	33'	24	22	Female
	11.5	2	0.18	109	80	50'	29	26	Male
TABLE V	15.4	3	0.19	105	72	1 hr. 35'	33	31	Female
	8.3	3	0.16	101	56	2 "	45	44	Male

DIGITALIS CONT'D

	WEIGHT IN KILOS	DOSE IN C.C.	DOSE PER KILO	NORMAL HEART RATE	DIGITALIS HEART RATE	TIME FOR DROP	AMOUNT OF DROP IN BEATS	PER CENT SLOWING OF HEART	
TABLE VI	7.1	.071	0.01	156	116	1 hr. 10'	39	24	Female
	17.2	.172	0.01	98	84	1 " 10'	14	14	Female
	17.4	.174	0.01	110	92	1 " 42'	18	18	Male
	16.7	.167	0.01	97	85	1 " 07'	12	12	Male
TABLE VII	10.94	.208	0.02	137	114	1 " 10'	33	24	Male
	15.9	.318	0.02	121	100	1 " 05'	21	21	Male
	21.8	.438	0.02	124	100	27'	24	19	Male
	26.8	.536	0.02	96	76	1 " 30'	20	20	Male
	10.7	.214	0.02	120	88	1 " 30'	33	33	Male
	9.3	.186	0.02	108	84	56'	24	22	Female
	9.8	.196	0.02	115	93	56'	22	20	Male
	14.5	.99	0.02	130	98	45'	32	17	Female
	17.5	.35	0.02	122	96	1 " 05'	26	22	Female
	10	.2	0.02	98	82	59'	16	16	Female
	17.3	.356	0.02	112	90	1 " 13'	22	19.6	Female
	23.6	.46	0.02	102.5	87	15'	15	15.2	Male
	5.9	.116	0.02	118	90	45'	28	23.6	Male
	14	.980	0.02	104	84	1 " 10'	20	19.2	Male
	18.8	.376	0.02	118	90	1 " 05'	28	23.8	Female
TABLE VIII	20.8	.654	0.03	128	88	1 " 55'	40	31.2	Female
	10.36	.310	0.03	118	92	1 " 35'	26	23	Female

Another series of animals were injected with increasing doses until toxic symptoms appeared. Doses which were used were as follows: from $\frac{1}{2}$, 1, 2, and 3 c.c.

Doses of 0.03 c.c. or more per kilo body weight usually elicited toxic symptoms such as nausea and vomiting. This led us to think that the therapeutic dose, as far as dogs are concerned, must lie somewhere between 0.01 c.c. and 0.03 c.c. per kilo, which we found to be approximately 0.02 c.c. per kilogram body weight of dog. When this dose was used the percentage drop was rather constant and no toxic symptoms appeared. The 0.01 dose per kilo body weight did not produce the maximum effect. In seven dogs injected with the 0.02 c.c. of the tincture per kilogram body weight the percentage drop varied only 5 per cent. In eleven other dogs used, the dose was varied from 0.01 to 0.03 c.c. of tincture per kilo. In these experiments the percentage drop showed greater variations.

DISCUSSION

The method presented is based upon a therapeutic standard and requires very little time and expense.

From the results one is justified in saying that standardization may be secured from the use of a few dogs. After about one week these dogs can be used again for standardization, thus eliminating a great expense for new animals.

The most constant percentage drop, produced by the 0.02 c.c. per kilo body weight of dog, does not produce toxic effects and is well within the therapeutic limits. Too small doses do not give so constant a result, too large doses, because of the nausea, do not give reliable results. A medium sized dose (0.02 c.c. per kilo) presents a variation of about 5 per cent on either side of a 20 per cent reduction of heart rate. The method is presented tentatively with the belief that it is reliable within reasonable limits, and it can be used in many instances where more laborious methods are unavailable. With a single dog, without injuring him, one may closely approximate the strength of a given preparation since 0.03 c.c. per kilo of a standard tincture will usually cause nausea and vomiting and 0.01 c.c. will cause considerable slowing of the heart rate. If a group of dogs be kept for the standardization, very dependable results can be obtained. This method of administering digitalis is very impressive as a class exercise.

PROPOSED MODIFICATION OF THE KAISERLING METHOD FOR PRESERVATION OF SPECIMENS FOR DISPLAY PURPOSES*

By Richard Lundquist, and H. E. Robertson, M.D., Rochester, Minnesota

IN April, 1924, this proposed method for preserving gross specimens was presented before the International Association of Medical Museums. Further experience with the technic, as given, has only strengthened our confidence in its efficiency, and fully justifies this second report.

Like most museum workers, we had formerly considered that the Kaiserling method, as published in 1899, was by far the most reliable method available for use. However, the Kaiserling method was rarely entirely satisfactory, having certain definite objectionable features, as follows:

1. A lack of penetrative power, which has often resulted in too long an exposure to the fixing solution in an attempt to obtain proper fixation. The color return in these specimens following the use of alcohol would not be as good as in those which had been exposed to the fixing solution for a shorter period.

2. The specimen showed an undue amount of distortion, even after being carefully arranged in the fixing solution. When large numbers of specimens are handled, it is almost inevitable that a certain number will be improperly placed in the fixing solution. It was found practically impossible to correct these distortions after the specimens had once become hardened.

3. Further hardening and stiffening of the tissues in the alcohol used for the restoration of color.

4. The extremely uncertain permanency of color preservation.

The first two objections were considered to result from too strong a percentage of formaldehyde in the fixing solution. This hardened the outer surface of the tissue exposed to the fluid to such an extent as to prevent proper penetration of fluid. In an attempt to overcome these difficulties the following formula was devised, and the use of alcohol thereby eliminated.

SOLUTION 1, FIXING SOLUTION

Potassium acetate	850 gm.
Potassium nitrate	450 gm.
Chloral hydrate	800 gm.
Formaldehyde (40 per cent gas)	4,445 c.c.
Water	40,000 c.c.

It is important that the specimens be placed in the fixing fluid as soon as possible, and that there be a comparatively large volume of this fluid. The

volume should be at least from ten to twelve times greater than that of the specimens. To make this possible, we use a stoneware crock with an inside diameter of 21 inches, an inside depth of 12 inches and a capacity of 66 liters (16.5 gallons). This crock gives the volume desired, and also furnishes the wide diameter which is very convenient when a number of specimens are prepared simultaneously. Formulas as given are the correct amounts for these crocks.* Specimens should not be allowed to lie against each other, or against the bottom of the container. To avoid this, we suspend the specimens by a string attached to a cork which has been previously boiled in paraffin. Care must be exercised to attach the string in such a manner as to allow the specimen to assume its natural shape. The use of the cork prevents the specimens from lying on the bottom of the jar, and the suspended specimens do not crowd each other. This procedure further facilitates the finding of any individual specimen when desired. The specimens should be thoroughly fixed in the first solution. The time required for this will vary with different specimens, and the worker will have to use his personal judgment. A good plan is to allow at least from twelve to twenty-four hours for thin membranes, such as the intestines, and forty-eight hours or more for slices of liver, spleen, kidney, and so forth. Specimens may be tested by using gentle pressure to see if any unfixed blood will exude from cut surfaces. However, the danger of loss of color, owing to too long exposure to fixing solution, is not nearly as great as with the original Kaiserling method. In any case in which it is desirable to preserve an unusually large specimen in its original size, gashes should be cut into it, not over 1.5 to 2 inches apart, to allow for penetration of fixative. These may be stitched together later, if desired. After the specimens have been properly fixed, *they should be thoroughly washed in running water for at least from four to six hours.* It is very important that all formaldehyde be removed from the specimens before final preservation. After washing, the specimens are trimmed. All excess fat is removed, and all cut surfaces are resurfaced, by removing a thin layer with a long, sharp knife, to make them smooth and also to remove the upper layer of tissue from which the blood pigments have been extracted. They are then placed in Solution 2.

SOLUTION 2, PRESERVING SOLUTION

Potassium acetate	4,000 gm.
Chloral hydrate	2,000 gm.
Glycerin	4,000 c.c.
Water	36,000 c.c.

Two crocks containing this solution are used. The specimens are allowed to remain in the first crock for twenty-four hours, and are then transferred to the second, and allowed to remain in this until they are to be mounted. When the solution in the first crock becomes dirty, or has an odor of formaldehyde carried over into it, it is discarded, the solution in the second crock is transferred to it, and a fresh solution made in the second crock. For the final mounting fluid, the same formula as given in Solution 2 is used. We have

*The crocks were obtained from the Red Wing Union Stoneware Co., Red Wing, Minnesota.

not found it necessary to use any further chemical to prevent growth of bacteria, moulds, fungi, and so forth. To date we have found no growth in specimens preserved in this solution. However, it may be possible that under different conditions and a different climate some trouble might arise. The addition of a preservative to the mounting solution, in time has a harmful action on the color of the specimens. This applies especially to the use of formol or phenol. With the original Kaiserling formula, arsenious acid, suggested by Delépine, was much better than either of these.

The solution used for filling the jars containing mounted specimens should be perfectly clear. We formerly filtered it through paper, using charcoal. This was a slow and tedious procedure, and often when a considerable amount had been filtered, the paper would break and necessitate refiltering the whole amount. A much simpler method is to use a 10 by 2 inch Berkfeld V filter cylinder, in a detachable nickel-plated metal cylinder, adding powdered animal charcoal, and filtering under a low positive pressure.

There is no new principle involved in this method. The fixing solution is the same as Kaiserling's fixing solution, except for the addition of the chloral hydrate and the lowering of the percentage of the formaldehyde used. The addition of the chloral hydrate apparently prevents the loss of color so common with the original Kaiserling method. Chloral hydrate has been used by Heidenreich, Jores-Köln, Frost, Klotz and Maclachlan, Haythorne, and Klotz and Coburn in their formulas, but all of these authors have left out the chemicals suggested by Kaiserling, and it is these which we believe have an important part in the preservation of the colors. The second solution is the same as Kaiserling's third solution, except that the percentage of potassium acetate and glycerin is reduced, and chloral hydrate is added.

REFERENCES

[1]Delépine, Sheridan: On the arsenious acid-glycerin-gelatin ("arsenious jelly") method of preserving and mounting pathological specimens with their natural colours, and on the use of new forms of receptacles for keeping museum specimens, Internat. Assn. Med. Mus. Bull., 1915, v, 69-72.

[2]Frost, R. L.: A new and inexpensive solution for the mounting of museum specimens, Internat. Assn. Med. Mus. Bull., 1913, iv, 41-42.

[3]Haythorne, S. R.: An efficient and inexpensive permanent mounting fluid for gross specimens, Internat. Assn. Med. Mus. Bull., 1915, v, 66-68.

[4]Heidenreich, L. L.: O nailutschei zhidkosti dlya sokhrenenyia anatom. preparatov, Russk. Vrach., St. Petersburg, 1903, No. 16.

[5]Jores-Köln: Demonstration einer zweckmässigen Modifikation des Knoservierungsverfahrens, Verhandl. d. deutsch. path. Gesellsch., 1913, xvi, 357-358.

[6]Kaiserling, Carl: Ueber Konservirung und Aufstellung pathologisch-anatomischer Präparate für Schau- und Lehrsammlungen, Verhandl. d. deutsch. path. Gesellsch., 1899, ii, 203-217.

[7]Klotz, Oskar, and Maclachlan, W. W. G.: A modified Jores' method for the preservation of colors in gross specimens, Internat. Assn. Med. Mus. Bull., 1915, v, 59-60.

[8]Klotz, Oskar, and Coburn, W. H.: A mounting fluid for gross specimens in their natural colors, Internat. Assn. Med. Mus. Bull., 1916, vi, 51-53.

MODIFIED GRAM STAIN (RUHLAND)*

By Arthur H. Sanford, M.D., Rochester, Minnesota

THERE are many modifications of Gram's differential stain for bacteria. In fact, probably very few bacteriologists use the stain first described by Gram. The essential characteristic of this important procedure is the behavior of the organism when stained with one of the violet dyes: gentian-violet, methyl violet, or crystal violet, then treated with iodine in Gram's iodine solution (one-fifteenth strength Lugol's solution), and decolorized with ethyl alcohol. Acetone decolorization has been very satisfactorily substituted for alcohol by Lyons.[1]

The following formula was given to me fourteen years ago by G. C. Ruhland, at that time director of the laboratories of the Milwaukee Health Department, Milwaukee, Wisconsin, and is now published with his permission. This modification of Gram's stain was entirely original with Dr. Ruhland. I have used it for more than thirteen years in the section on clinical laboratories of the Mayo Clinic, and it is the most satisfactory modification of Gram's stain that I have ever used or seen. Our only modification has been the substitution of acetone for grain alcohol for decolorization. Crystal violet (hexamethyl pararosaniline chloride) can be obtained in the most nearly pure form of any of the violet dyes. Gentian-violet is merely a mixture of penta- and hexamethyl derivatives. Methyl alcohol, the solvent for the dye, is also an excellent mordant. The solution of crystal violet in methyl alcohol is absolutely stabile, a desirable property in any modification of Gram's stain. The safranine counterstain is an excellent contrasting color for the observation of Gram-positive organisms; the Gram-negative organisms are stained a brilliant red.

Formula for Ruhland's Modification of Gram's Stain

SOLUTION A

Crystal violet	2 gm.
Methyl alcohol (special, absolute purity)	100 c.c.

Stain the fixed slide about thirty seconds.
Rinse in tap water.
Then flood the slide with Solution B for thirty seconds or one minute.

SOLUTION B
Gram's Lugol Solution

Iodine	1 gm.
Potassium iodide	2 gm.
Distilled water	300 c.c.

Rinse the slide with tap water and flood it with acetone, C.P. until no more color can be removed; decolorization of a thin preparation takes place instantly.
Counterstain with Solution C.

SOLUTION C

Safranine	1 gm.
Distilled water	100 c.c.

Wash.
Dry.

AN IMPROVED SELF-FEEDER FOR RATS*

By Ralph Hoagland, B.Agr., and Oliver P. Clipper, Washington, D. C.

IN a previous article,† one of the writers described a self-feeder for rats
for use in feeding tests when an accurate record of the food intake was
desired. That type of feeder, with slight modifications, has been used in this
laboratory during the past three years in feeding tests with more than 2200

rats and it has answered the purpose reasonably well. Experience has shown, however, that it has certain faults, and we have designed a new self-feeder which has proved to be much more satisfactory than the old one.

Scale drawings of the new feeder are shown in Plates I and II. It consists of two parts; viz. (1) Body, as shown in Plate I, and (2), Screen, as shown in

PLATE 2

United States Department of Agriculture.
Biochemic Division — Bureau of Animal Industry.
Title — Self-feeder for Experimental Rats.
Material of Construction — Tinned Copper.
Assembly — Screen, Shown in Plate II, Sets
On Shoulders of Body, Shown in Plate I.
Scale of Construction — 1"=1"
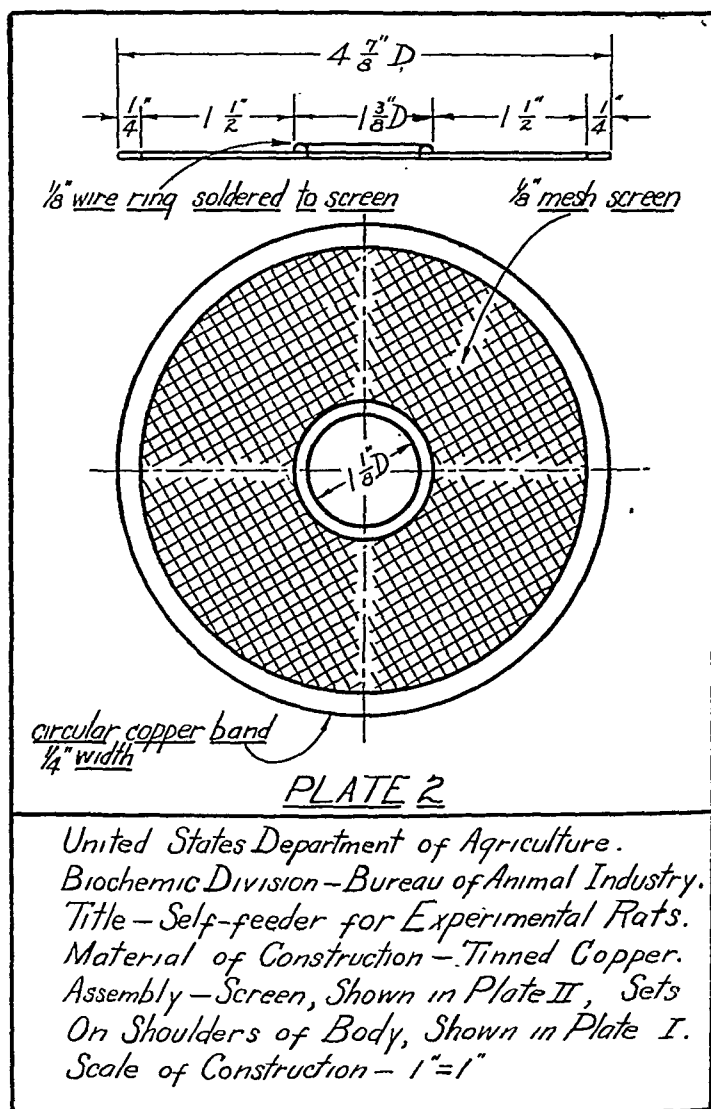
Plate II. The body consists of a cylinder in which is soldered an inverted truncated cone that serves as a feed receptacle. The body is made of tinned sheet copper and all joints are soldered. The copper at the top and bottom of the cylinder is crimped so as to make the feeder stronger. The screen is made of galvanized iron wire with a copper band around the circumference. The feed hole in the center of the screen is reinforced with a galvanized iron ring which projects above the surface of the screen and serves to prevent feces

from dropping into the feed receptacle. The feeder is simple in construction and substantial.

The feed receptacle or hopper has a maximum capacity of 150 grams, but in practice it has been found best to add approximately 100 grams of feed so as to leave some space between the feed and the screen. This space permits any feed which the rat has carried on to the screen to drop back into the hopper. The feed hole shown in Plate II has proved satisfactory with rats ranging in size from 40 to 200 grams, but a slightly larger opening is required for heavier rats.

The improved self-feeder needs relatively little attention. A rat can easily reach sufficient feed for a period of two or three days without the feeder being attended to, but in practice the feeders are examined daily, except Sunday, and the feed in the hopper is leveled off when necessary. This feeder has proved very satisfactory under a variety of conditions and we intend to replace all of our old type feeders with the new one as rapidly as practicable.

AN IMPROVED DEVICE FOR THE DETERMINATION OF DEXTROSE IN URINE BY FERMENTATION*

By Raymond Szymanowitz, B.S., Newark, N. J.

IN the determination of dextrose in diabetic urine by fermentation, using the Einhorn saccharimeter, dissolved or occluded gases are frequently evolved and collect in the graduated limb of the instrument. This is especially the case when the fermentation is hastened by employing a temperature slightly higher than that of the room.

Lange† found that the small amounts of nitrous and nitric acids in the urine react with urea, amino acids, and ammonia to form nitrogen. A gas so generated, together with the liberation of any entrapped air in the urine, would obviously contribute to the inaccuracy of this method.

To compensate for the solubility of carbon dioxide, the first division of the scale on the graduated limb of the saccharimeter represents a sugar content of slightly less than .25 per cent. For this reason, in the absence of confirmatory tests, air and nitrogen alone might erroneously indicate a sugar content approaching .25 per cent in a dextrose-free urine. In saccharinous urine the results obtained would be higher than the true content. To eliminate the error described herein, the following method and device has been devised.

An Einhorn saccharimeter equipped with a two-way stopcock on its graduated limb, is filled with the urine-yeast solution in the regular way. Upon completion of the fermentation, the saccharimeter is connected with a graduated U-tube, filled with an alkali or any suitable liquid absorbent of carbon dioxide,

through a ground joint as illustrated. The glass tubing carrying the stopcock is of capillary stock and not unlike that employed in general gas analysis.

To free the capillary tubing, as well as the stopcock, of air, the cock is so turned as to permit the escape of a few drops of the contents of the U-tube through the drain tube illustrated. The cock is then turned to a neutral position while the rubber bulb is connected to the shorter limb of the saccharimeter by means of a rubber stopper carrying a small piece of glass tubing.
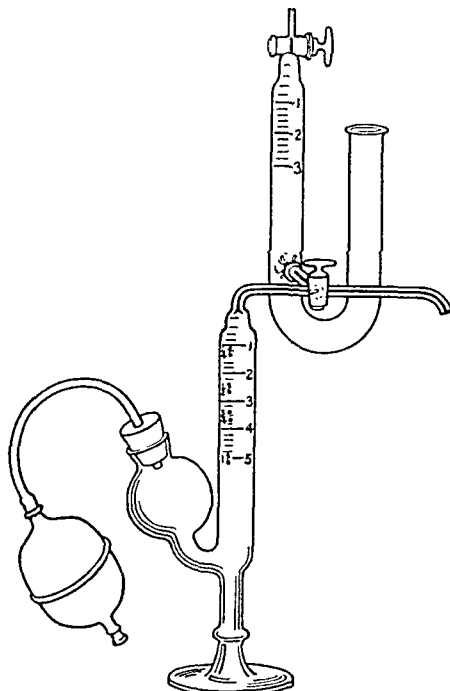
Fig. 1.

By the simultaneous application of gentle pressure to the bulb and proper adjustment of the stopcock, the gases contained in the saccharimeter may be slowly driven into the U-tube where absorption of the carbon dioxide takes place. The comparatively insoluble gases, i. e., nitrogen and oxygen, are found in the graduated limb where they may be read directly as to volume in cubic centimeters. The difference in the readings obtained before and after absorption represents the actual amount of carbon dioxide liberated in the fermentation process in excess of the carbon dioxide in solution in the urine and which was taken into consideration during the calibration of the saccharimeter.

The Journal of Laboratory and Clinical Medicine

EDITORIALS

Renal Function In Clinical Medicine

THE kidneys have an abundant blood supply, the renal arteries taking off directly from the abdominal aorta and being quite short. The arterial system leads eventually to the glomeruli of the kidneys where the arterioles break up into large capillary tufts. The vessels leading from the glomeruli are distinctly smaller than the afferent ones. This favors the idea that the blood in passing through the glomerulus loses a large proportion of its water. The arterioles leading from the glomeruli break up again into a second capillary network distributed around the convoluted tubules. This double capillary system is quite unique among the organs of the body and justifies in part the assumption that while water and perhaps other substances pass out through the glomeruli, the excretion of certain other constituents of the urine is a function of the convoluted tubules.

The prevailing theory has it that fluid passes out through the capillary

walls in the glomeruli, through the very thin Bowman's capsule purely by physical processes, and that this transudative fluid contains its constituents in approximately the same concentration as that of the blood plasma. The glomerulus acts as a semipermeable membrane allowing passage to water and most of the crystalloid substances but holding back the colloids. As this very dilute urine passes the cells of the convoluted tubules it is altered by the resorption of some of its constituents through the epithelium and back into the blood stream. Aschoff divides the urine excretory system into three sections, the glomerulus or filtration apparatus, the proximal convoluted tubule or resorption apparatus, and the loop of Henle, which serves as a pressure regulator.

The colloidal chemists explain the resorption of water in the primary tubules on physical grounds. Fischer believes that blood rich in carbon dioxide or venous blood has a tendency to swell or absorb water, while blood poor in carbon dioxide such as arterial blood has a tendency to give up water or shrink. The arterial blood in the glomeruli gives up water readily while the venous blood in the second set of capillaries tends to absorb water.

The resorption of water explains the concentration of urine but it does not explain the alteration in proportion of the various elements. Sodium chloride is concentrated to double the plasma strength, while urea is concentrated about forty times. In explaining this, Cushny divides the constituents of the blood plasma into threshhold bodies and nonthreshhold bodies. Dextrose, chloride and sodium are threshhold bodies and are excreted only when their concentration in the blood exceeds a certain percentage. Threshhold bodies are necessary for vital processes and are retained unless in excess. On the other hand, substances which are of no use to the body, such as urea, are excreted when present even in small amounts and in this way have no appreciable threshhold of excretion.

According to the colloidal theory, substances with threshhold value pass through the glomeruli but are resorbed along with water through the tubular epithelium.

Aschoff maintains that in such a condition as mercury poisoning, mercury is excreted through the glomerulus and it is only during its resorption through the tubules that damage to the latter occurs. Gil-y-Gil has demonstrated that in experimental uranium poisoning, the epithelial cells damaged during resorption acquire a tolerance to the poison. Lethal doses may be subsequently given with impunity, the uranium being excreted in the urine instead of being taken up in the cells. This gives us an insight into one phase of the production of chronic nephritis. The epithelial cells become less permeable, if we may use this term, to the substances which have passed out through the glomeruli and do not resorb them as they should. This is probably true of water as well as of the substances in solution. As a result the concentrating ability of the kidneys becomes damaged.

We have been accustomed to describe parenchymatous nephritis and glomerular nephritis. In the one the damage appears to have been chiefly tubular, in the other glomerular, but both systems are more or less involved in either type and the clinical differentiation is often difficult or impossible. The word nephritis presupposes an infection or inflammation of the kidneys. Nephrosis

is a designation applied to those types of renal pathology in which there is no evidence of infectious origin and in which the cause appears to have been toxic. This applies particularly to those cases with predominant involvement of the tubular epithelium. Parenchymatous nephritis is characterized by degeneration of the epithelium lining the uriniferous tubules with little or no involvement of the glomeruli. The kidney is slightly enlarged, the cortex broadened, and an excess of fat deposit is found in the cells of the tubules. As a general rule there is no cellular infiltration of the connective tissue suggestive of localized infection so that we may consider this condition a nephrosis. This is the type of picture observed in mercuric chloride poisoning.

In acute and chronic glomerulonephritis the gross appearance resembles that of parenchymatous nephritis. The tubules are involved in greater or less degree, the glomeruli are swollen and show an increase in the number of nuclei dependent on multiplication of both endothelial and epithelial elements and in the advanced cases crescentic mass of desquamated cells occupies the capsular space. These are usually inflammatory changes. Glomerular involvement does not undergo such complete recovery as does the parenchymatous type. The vascular tufts become adherent in places to the capsule and destruction of portions of the glomerulus results in partial hyalinization.

The interstitial connective tissue almost invariably undergoes proliferation and it was customary formerly to distinguish a parenchymatous and an interstitial type of nephritis, depending upon the degree of connective tissue formation. Weigert has shown however, that chronic parenchymatous lesions are nearly always associated with interstitial growth. He interprets the connective tissue proliferation as an attempt to fill in the defect caused by the destruction of parenchyma, that is, a replacement fibrosis.

The secondary contracted kidney follows extensive parenchymatous destruction and connective tissue overgrowth and we may look upon this as the end stage of a chronic glomerulonephritis. The scar tissue in the kidney has a patchy distribution.

The primary contracted kidney or red granular kidney differs in many essentials from the types of kidneys just described. In the red granular kidney we observe bands or wedge-shaped areas of fibrous tissue enclosing atrophic tubules and obliterated glomeruli, alternating with active parenchyma. The glomeruli are small, hyaline and surrounded by a thickened capsule. They have suffered total destruction. In other portions of the kidney they are normal. The arterial walls in the red granular kidney are always affected. The elastic layer becomes thickened and the intima undergoes fatty degeneration. These degenerative lesions are undoubtedly a phase of arteriosclerosis and the vascular lesion or arteriosclerosis is probably the chief factor in the production of the primary contracted kidney. The vascular changes are followed by nutritional disturbances which endanger particularly the glomerulus. After their destruction the tubules atrophy from disuse. Thus we see that the renal damage in all forms of nephritis involves both the glomeruli and the tubules, and the variations in the different types are only relative. Even in the arteriosclerotic or

primary contracted kidney both systems are involved. It is apparently only in true nephrosis that the glomeruli remain relatively free from damage.

We can, therefore, readily understand the difficulty of attempting to localize renal injury in the living patient, whether in the tubules or in the glomeruli. It is far safer and more correct to speak of acute or chronic nephritis with or without hypertension, thus describing the renal pathology as a whole. This attitude has been urged particularly by Christian in this country and by Yores and others in Europe.

The fallaciousness of simple qualitative urinalyses is known to all. The value of functional tests is conceded. The interpretation of their significance is on the other hand, not always above question. We no longer think of the salicylic acid test as a measure of the permeability of the glomeruli, the methylene blue test as a measure of the osmotic function of the epithelial cells, and the phloridzin test as a means of determining the secretory ability of the renal epithelium. Even with those tests which are in general use today, it is hazardous to venture concrete hypotheses with regard to their modus operandi in the kidney.

In terms of the preceding discussion we may, however, at a certain risk of accuracy, visualize the reaction of the kidneys to the commonly used functional tests somewhat as follows:

Disease of the kidneys affects the body either by allowing substances to pass out which should be retained, or by retaining substances which ought to pass out. Of the substances which should be retained but do escape, albumin is of chief importance. Of those which should be excreted but are retained, those commonly measured are uric acid, urea, and creatinin. Either the glomerular membrane becomes less permeable to them or they are resorbed in the tubules. The former seems the more likely. These are nonthreshhold substances and normally are not resorbed. Moreover, observations indicate a diminution rather than an increase in the absorptive capacity of the tubules.

Phenolsulphonephthalein is an organic dyestuff toward which the body reacts as it does toward urea. Undoubtedly it is excreted through the glomerular epithelium and normally is not resorbed to any great extent in the tubules. Like urea, in chronic nephritis it probably does not pass as readily through the glomeruli. The reaction of the kidneys to phenolsulphonephthalein is proportionately so similar to their reaction to urea and other nitrogenous substances that the phthalein test has come to be used as a measure of the capacity for excretion of nitrogenous substances.

We cannot at present explain with entire satisfaction the inability of the damaged kidneys to concentrate those crystalloids which do pass the glomerulus. The fluid passing through the glomeruli presumably contains those crystalloids which are not materially obstructed in about the same concentration as in the blood. Failure to concentrate is then presumably due to some change in the tubular epithelium. We have seen that the chronically poisoned epithelium becomes less permeable to certain substances. It is possible that the failure to concentrate substances such as sodium chloride is due to lessened resorptive capacity for water.

We are not so much interested in sodium chloride as an excretory product as we are in the kidneys' ability to concentrate.

It is usually stated that the urea excretion is an index of glomerular function while salt and water excretion is an index of tubular function. The two latter are threshhold bodies. From the foregoing we can see how in a general way this may be true.

We should state parenthetically that the prevailing concept of urinary excretion and concentration, based chiefly on the recent work of Cushny, that concept on which our discussion is based, is not without its weak points. Thus MacNider has demonstrated columnar epithelium in the glomeruli of the 'possum.

Certain generalizations have been made from functional studies, which, while in the main correct, have important exceptions and it is our desire briefly to direct attention to certain of these. It has been stated for example that the examination of a single specimen gives no information regarding the functional capacity of the kidney. It is true that little information is gained from a single urinalysis and that when its specific gravity is low, we have no indication of the functional capacity of the organ. But when the specific gravity is 1020 or above we may be confident that the concentrating ability of the kidney is reasonably good, and that nitrogenous substances are being excreted by the kidney in essentially a normal manner. Thus a single specimen with high specific gravity is of definite value. Of course it is necessary to rule out the presence of sugar.

It has been stated that when the phthalein excretion is high we may be confident that there is no nitrogen retention in the blood. This is not always true. The particular exception is to be found in acute nephritis. The kidney is able to eliminate phenolsulphonephthalein, urea, etc., in normal concentration but the trouble lies in the water excretion which is so greatly impaired that not enough water can pass through to prevent the damming back of urea in the blood.

A high specific gravity does not necessarily indicate normal ability to concentrate urine. We find lack of flexibility in the two-hourly renal test, the variation in specific gravity throughout the day being less than ten points, showing distinct fixation of specific gravity at a high level. This is particularly apt to occur in parenchymatous lesions and in cases of long-standing focal sepsis.

A tendency toward fixation of specific gravity at a high level may appear in normal individuals who from habit have a low water intake. This observation is of help in directing their daily routine in that with a greater fluid intake they develop a normal type of reaction.

No one test is sufficient in all cases and no study of renal function even in the mildest nephritic is sufficiently complete unless all of those tests of proved value have been made and correlated. No single functional test is sufficient for the recognition of early pathology. This is due chiefly to two factors: first, excretion for some substances may be normal while impaired for others, and second, even in the normal there is considerable fluctuation in the response, and multiple checks must, therefore, be applied.

—W. T. V.

DR. JOHN A. KOLMER
Philadelphia, Pennsylvania
President, American Society of Clinical Pathologists
1924-1925

The American Society of Clinical Pathologists

On to Philadelphia!

History of the American Society of Clinical Pathologists

Introduction.—An organization that is just approaching its third birthday can hardly lay claim to an historical retrospect. So many dramatic events, however, are crowded in the Society's young life that a brief recital of its genesis and growth will probably strike a responsive chord among the members and friends who have watched with interest the meteoric rise of the American Society of Clinical Pathologists.

Predisposing Causes.—The time was ripe for the formation of such an organization. There were many clinical pathologists scattered throughout the medical communities of this country, each more or less isolated from his colleagues in this specialty, trying in his own way to meet the problems that faced him and longing for communion and fellowship with kindred souls for counsel in his difficulties. Above all, it was in the scientific field that he looked for help, guidance or inspiration. Like his confreres in other specialties, he naturally yearned to meet with his peers, at periodical reunions, to discuss the results of the newest research or improved methods in laboratory investigation. None of the existing societies, narrowing their activities to one particular branch of clinical pathology and catering chiefly to college professors, quite satisfied the longings of the practical laboratory man. He desired to meet with men like himself engaged in the professional field, in daily contact with patient and clinician, and confronted with problems similar to his own. In addition to the thirst for knowledge that would aid him in the diagnosis of disease, there was another circumstance that made the formation of a national organization an inevitable outcome. The existence of laboratories, some of them directed by incompetent laymen, advertising their skill and their prices in reputable medical

journals, was a menace that threatened the future continuance of ethical prac-
titioners in this specialty. It was, therefore, a foregone conclusion that sooner or
later, a union of the forces striving for a high plane of practice in this field
would take place.

Modern historians give little weight to the superman theory ascribing events
and occurrences to preexisting causes and external conditions.

Exciting Cause.—So it was with the inception of the American Society
of Clinical Pathologists. It so happened that circumstances in one of the
western states furnished a favorable nidus for this idea to germinate and evolve
to a successful fruition. And now we shall for the nonce forsake the cold im-
personal viewpoint and turn to the human interest side of the story of the
A. S. C. P.

On March 14, 1921, a group of five clinical pathologists, all in active
practice of their specialty in the city of Denver, Colorado, met in the labora-
tory of one of their number to consider the details of a program to be pre-
sented before the local County Medical Society on blood chemistry determina-
tions. One of the group who issued the call for this first meeting of the
Denver laboratory men had been asked by the Program Committee of the
Medical Society to suggest the names of the essayists for the blood chemistry
symposium. Fortunately, the esprit de corps of the Denver Clinical Pathol-
ogists always has been ideal and their mutual relations the most friendly and
cordial. It was but natural, therefore, for them to get together on this occasion
and select from their number the speakers who were to present the various
angles of blood chemistry in relation to disease. The gathering for this special
purpose logically led to the desire for more meetings and thus the Denver
Society of Clinical Pathologists was formally launched April 1, 1921.

It was not long before similar friendly relations with colleagues in the
neighboring cities of Colorado Springs and Pueblo led to their being invited
to participate in these meetings. On June 21, 1921, the name of the organiza-
tion was changed to the Colorado Society of Clinical Pathologists. All the
clinical pathologists of the state were enrolled as members and meetings were
held quarterly. At these gatherings, besides the presentation of scientific
papers, informal discussions were held on the status of the clinical pathologist
and his relation to the clinician. The situation was indeed deplorable. The
clinical pathologist was coming to be regarded as a mere technical assistant,
not on a par with other specialists. This condescending attitude was greatly
fostered by the unseemly advertisements in medical journals tending to make
laboratory diagnosis a purely commercial adjunct.

Other Foci of Infection.— Again assuming the historian's viewpoint of
cause and effect, it is interesting to note that under similar circumstances a
State Society of Clinical Pathologists had been formed in Texas prior to the
one in Colorado. Is it that the great open spaces of the West and Southwest
where habitations are far apart and the herd instinct more pronounced that
conditions favor the spirit of neighborliness more than in the congested East?
It is idle to speculate on the etiology or lapse into generalization which may
prove illogical for lo, about the same time we find Ohio organizing a State

DR. PHILIP HILLKOWITZ
Denver, Colorado
Past President, 1922-1923

DR. WM. CARPENTER MACCARTY
Rochester, Minnesota
Past President, 1923-1924

DR. FREDERICK E. SONDERN
New York City, N. Y.
First Vice-President

DR. WARD BURDICK
Denver, Colorado
Secretary-Treasurer

Pathological Society. Friendly correspondence with these state organizations cheered each one on in its work.

Period of Polemic.—With the encouragement of the sister society of Texas, the officers of the young Colorado Society of Clinical Pathologists launched their famous campaign against the advertisement of laboratories in medical journals. With the enthusiasm characteristic of youth, numerous conferences were held and plans laid for fighting the evil. Courteous letters of protest were sent to the trustees of the American Medical Association calling attention to the unseemly advertisements in the Journal as unworthy of the good character of the official organ of the medical profession of America. Resolutions decrying the acceptance of advertisements from commercial laboratories by medical journals were drafted and submitted before the Medical Society of the City and County of Denver which passed it unanimously, other medical societies later following the example.

Letters of mild protest were sent to honorary consultants whose names figured in the advertisements of a laboratory on the "chain of stores" plan, pointing out the anomaly of their position as teachers in medical schools lending their prestige to a commercial concern. All this formed a lively chapter in the career of the newly organized state society, the battle for the right spurring it on to greater efforts, the resistance of the opposition only stimulating the quicker coursing of the blood.

Preliminaries to Organization.—The need of a national body to take up the fight was sorely felt. It transcended, for a while, the yearning for the scientific purpose of such an organization. How can a few humble and obscure pathologists, in a provincial town in the far away West set the machinery in motion to get a national organization started? Yet the matter was quite simple. The conditions were ripe. The determining factors were all there. Chance and a little initiative got the wheels a-going. Late in 1921, a letter and questionnaire were sent out on the letterheads of the Colorado Society to all the colleagues listed in the American Medical Association Directory under the designation CP with an asterisk, signifying that they limited their practice to Clinical Pathology. The culling of the names, in itself a task, was distributed among the members and in a trice the list was complete. A copy of the circular may be of historical interest.

"The formation of societies of clinical pathologists in various cities and states has naturally led to a desire for the creation of a national organization.

"With this object in view we are addressing a circular letter and questionnaire to all physicians who specialize in this branch of medicine.

"The necessity for an organization of this kind is very urgent in view of the strong tendency to commercialize our specialty and to degrade the pathologist to a mere technician.

"The scope of the proposed organization would be to advance the scientific progress of Clinical Pathologists and to maintain the status of the Clinical Pathologist as a consultant in medicine on an equal plane with that of the internist or surgeon.

"It is proposed to form this organization at the next meeting of the American Medical Association at St. Louis, where problems relating to our specialty may be discussed personally and in detail.

"Any suggestions from you will be greatly appreciated."

DR. H. J. CORPER
Denver, Colorado
Member of Publication
Committee

DR. F. M. HUNTOON
Glenolden, Pennsylvania
Member of Committee on
Standardization of Lab-
oratory Reagents

DR. THADDEUS WALKER
Detroit, Michigan
Member of Standardization
Committee

DR. RUTH GILBERT
Albany, New York
Member of Standardization
Committee

DR. J. H. BLACK
Dallas, Texas
Member of Committee on
Standardization of Labora-
tory Reagents

DR. A. V. ST. GEORGE
New York City, New York
Member of Standardization
Committee

DR. GEORGE IVES
St. Louis, Missouri
Member of Publication
Committee

DR. WM. C. EXTON
Newark, New Jersey
Member of Publication
Committee

Attached was a questionnaire inquiring among other things whether the addressee would join a national society of Clinical Pathologists and whether he would attend the session of the A. M. A. at St. Louis where the proposed society would be organized.

To the four hundred and fifty letters that were sent out there came back one hundred and seventy-five replies, the great majority of which fervently advocated the formation of such a society and many of them promising to attend the convention and be ready for the call.

Heartened by the extraordinarily favorable response, steps were soon taken to issue a call for the convention. It was deemed advisable to make the time and place coincident with the meeting of the American Medical Association in order to insure a good attendance. Dr. George Ives of St. Louis having been among the first to respond to the questionnaire was asked to act as a Committee of Local Arrangements, and right loyally did he execute the duties allotted to him. A commodious room in the Missouri Baptist Hospital was, through his efforts, assigned as a meeting place for the clinical pathologists. At the proper time announcements of the forthcoming convention were sent to all the colleagues on our mailing list.

The First Annual Convention.—The fateful day of the meeting was drawing near. Arrangements had all been completed. It was with some fear and trepidation that the signers of the call awaited the portentous event. On Monday, May 22, 1922, at eight P.M., about forty men and a few ladies were assembled at the meeting place awaiting developments. Very few of those present were personally acquainted with one another and the atmosphere was somewhat tense and expectant. The meeting was called to order by Dr. Ives. In the minutes of the first convention are found his prophetic introductory remarks. "I have assumed the honor of calling to order what is destined to be the first successful national meeting of Clinical Pathologists."

In accordance with parliamentary procedure, he nominated a temporary chairman and on the latter's assumption of the chair, he also nominated a temporary secretary. The courtesy of these two offices was accorded to the Colorado delegates—Dr. Philip Hillkowitz and Dr. Ward Burdick, who had issued the call.

Parturient Pains.—After a brief exposition by the chairman on the purpose of the convention, a general discussion took place on the meaning of the term "clinical pathologist" which brought forth a variety of views, but helped to clear the atmosphere and pave the way for definite organization.

On motion of Dr. MacCarty of Rochester, a committee of five was appointed to carry out the purpose of the convention and submit a constitution and by-laws for consideration. After further extended discussion on the part of those present on the relation of the pathologist to the clinician, the session adjourned until the following morning. The differences of opinion on the matters under consideration left the delegates with an uneasy feeling and some misgivings as to the future fate of the proposal for a national organization.

The Birth.—The next morning was ushered in with a flood of sunshine. The visitors had rested from their travel and were in fine humor. Promptly

Dr. HERMAN SPITZ
Nashville, Tennessee
Member of Executive Committee

Dr. MURRAY C. STONE
Springfield, Missouri
Member of Executive Committee

Dr. A. H. SANFORD
Rochester, Minnesota
Member of Executive Committee

Dr. W. F. THOMSON
Beaumont, Texas
Second Vice-President

Dr. ERNST A. VICTORS
San Francisco, California
Member of Board of Censors

Dr. A. H. SCHADE
Toledo, Ohio
Member of Board of Censors

Dr. WILLIS P. BUTLER
Shreveport, Louisiana
Member of Board of Censors

at eleven o'clock, the temporary chairman opened the meeting and immediately called for the report of the Committee on Constitution and By-Laws.

In the brief interval between the sessions, the committee would have had to work all night drafting the instrument but the machinery of the organization had been so well oiled that typewritten copies of a constitution and by-laws were in the hands of the committee immediately after their appointment and met with their approval so that but few alternations were made. The members present seemed to think likewise, for without any discussion the acceptance of the constitution and by-laws was carried unanimously. The temporary officers were elected president and secretary, respectively, for the ensuing year; Dr. William C. MacCarty, of Rochester, Minnesota, was chosen as first vice-president and Dr. H. R. Brown, of Rochester, New York, as second vice-president. To lend auspicious augury to the new organization a program committee appointed by the Chair hastily arranged from the talent present an extemporaneous scientific session for the afternoon gathering. Dr. Ives spoke on "Streptococcus Veridans Endocarditis" and Dr. MacCarty on "The Diagnostic Efficiency of the Average Clinician with Reference to Breast Tumors," both addresses being liberally discussed.

By this time the members had become well acquainted with each other. A feeling of fellowship was engendered. At the close of the meeting everyone felt elated at the success achieved and filled with high hopes for the future of the American Society of Clinical Pathologists.

Consolidation of Organization.—The year of 1922-23 was devoted to building up and strengthening the organization. The number of charter members at the close of the first annual convention was one hundred and forty-five. With the aid of these members and through the efforts of the Secretary's office, applications for membership rapidly increased.

Education of the Profession.—Considerable efforts were put forth to elevate the status of the clinical pathologist. The trustees of the American Medical Association were again appealed to in our campaign against laboratory advertising. A communication protesting against the action of the Advertising Committee of the A. M. A. in permitting the insertion of objectionable advertisements was published in the Journal and helped to arouse a more sympathetic attitude of the medical profession toward the clinical pathologist.

Our members were kept apprised of our progress by means of Bulletins which carried detailed accounts of our fight for the Ethical Practice of Clinical Pathology together with other items of interest.

The morale of the Clinical Pathologists throughout the country was thereby raised to an incalculable degree. They felt as never before that they were specialists in an important branch of medicine, consultants, on a par with the internist and the surgeon.

As the year rolled on the enthusiasm of the members grew apace and they looked forward with pleasant anticipation to the next annual meeting.

Second Annual Convention.—Again it was deemed advisable to hold the convention at the same time and place as that of the American Medical Association scheduled for that year at San Francisco. On account of the great

distance from Eastern centers no large attendance was anticipated. Nevertheless over seventy members registered at the second annual convention of the American Society of Clinical Pathologists which was held June 25, and 26, 1923. The scientific program was very good, the chief subject of interest and discussion being the technic of the Wassermann reaction. Considerable interchange of opinion took place on the economic aspects of the practice of clinical pathology. This was occasioned, to some extent by the presence of several men affiliated with commercial laboratories who objected to the attitude of the American Society of Clinical Pathologists toward advertising. The Society, however, by a practically unanimous vote registered its approval of the code of ethics laid down in its by-laws.

During the course of the meeting the trustees of the American Medical Association invited a committee from the American Society of Clinical Pathologists to give their view of laboratory advertising which was accordingly presented to them in the proper light.

As a result of the campaign waged by the American Society of Clinical Pathologists, laboratory advertisements though not entirely eliminated have been censored to the extent that no fees are published or undue claims made.

The membership during the second year continued to grow. Clinical pathologists all over the country, recognizing the valuable services rendered their cause by the society hastened to enroll under its banner.

In the election of new officers, Dr. Wm. C. MacCarty of Rochester, Minnesota, was chosen president, Dr. John A. Kolmer, first vice-president, Dr. George Ives, St. Louis, Missouri, second vice-president and Dr. Ward Burdick was reelected secretary-treasurer.

The incoming president invited the society to meet the next year in Rochester, Minnesota, as guests of the Mayo Clinic. The invitation was heartily accepted.

Third Annual Convention.—The central location, the large and interesting scientific program, the opportunity to see the extensive laboratories of the Mayo Clinic, all combined to bring out a large number of members and visitors at the Rochester meeting which was held June 5, to 7, 1924. By this time many of the members were well known to one another and cordial greetings of good fellowship were exchanged. The hospitality of the Mayos in offering the members all the facilities of their wonderful plant was deeply appreciated by all present at the meeting.

The papers presented were of a high order and the round table discussions by experts in their respective fields were very instructive.

Nothing but science held sway at the third annual meeting. Business matters and economic topics which loomed large at San Francisco were relegated to the background. It was clearly the intention of the committee of arrangements to demonstrate that the American Society of Clinical Pathologists is primarily a scientific society and that the other aspects of membership in this organization, though likewise desirable, are secondary. All the visitors went away profoundly impressed and highly elated at the high scientific level at-

tained by the society and correspondingly proud of the privilege of belonging to such an organization.

Official Organs.—Tentative arrangements had been made prior to the Rochester meeting for the JOURNAL OF LABORATORY AND CLINICAL MEDICINE to publish matters of interest to our members. During the convention the opportunity was presented through personal interviews with the editor and the publisher of the Journal to make this excellent periodical our official organ. All the papers presented at the meeting together with the transactions were accordingly published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

Officers Elected at the Third Annual Convention.—At the Rochester gathering, the society elected Dr. John A. Kolmer, of Philadelphia, Professor of Clinical Pathology at the University of Pennsylvania, President; Dr. Frederick E. Sondern of New York City, a pioneer in Clinical Pathology, first vice-president; Dr. W. F. Thomson, Beaumont, Texas, second vice-president, and Dr. Ward Burdick was reelected secretary-treasurer.

The Present.—The approach of the Fourth Annual Convention finds the American Society of Clinical Pathologists in a flourishing and healthy state. The perilous period of infancy when the mortality is highest has been safely passed over; the seasons of storm and stress when it was battling for recognition as a dignified specialty, are also left behind. The society is now a lusty youth full of enthusiasm and filled with ideals for a useful future. Its membership now exceeding three hundred and representing the best men in this field in all parts of the United States is devoted to the cause of helping humanity by advancing the cause of scientific medicine through the aid of clinical pathology.

The Administration of the American Society of Clinical Pathologists

From a portable typewriter in a corner of a crowded laboratory in a physicians' office building to a full complement of office equipment in a suite of commodious quarters in a modern hospital—such is the story of the rapid rise of the secretariat of the American Society of Clinical Pathologists. In the early days of the society—if it be proper to indulge in reminiscences of a three-year-old organization—all the business of the new fledgeling was conducted in the interim of making laboratory examinations with a microscope in front and an incubator in the background. The correspondence was pounded out on a small portable typewriter, and records kept in paper files.

The phenomenal growth of membership and enormous increase in correspondence soon necessitated having clerical help, at first only during periods of stress but later on half time basis. The need for better equipment and more office space was soon apparent. Fortunately the Children's Hospital, with which our secretary is connected as pathologist, has extended to the society the free use of very roomy quarters in this institution adjoining the clinical laboratory.

In this office is installed the usual armamentarium of a modern business office including a set of files which hold, besides the correspondence, an elaborate

series of card indexes covering all the activities of the American Society of Clinical Pathologists. Every member is listed both alphabetically and geographically with data pertaining to his membership.

The office of the American Society of Clinical Pathologists has developed into an information bureau and clearing house. Its activities are manifold. Aside from keeping in constant touch with the membership on the progress of the organization, it answers the many inquiries that come in on society matters. It receives and answers correspondence from hospitals and colleges

Children's Hospital, Denver, Colorado, wherein is located the office of the Secretary of the American Society of Clinical Pathologists.

Secretarial Quarters. The office is furnished with all necessary business appliances for conducting the affairs of a national organization.

Miss Marie Boes, Assistant to the Secretary
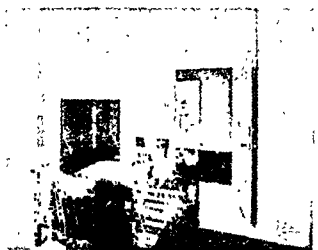
View of the offices of the American Society of Clinical Pathologists. Across the corridor are the laboratories of the Children's Hospital of Denver, Colorado

regarding available clinical pathologists and teachers in pathology, bacteriology and allied subjects. It likewise puts the clinical pathologist seeking a new location in touch with the hospital offering the prospect of a position. Much of the work of the publication committee is carried on in this office, also correspondence relative to clinical pathologists seeking membership, the arrangement of our annual program, matters consequent to our annual conventions, conducting detail work of various committees such as the Committee on Standardization of Laboratories, etc. With each year the functions of the

secretariat have been broadened so that at the present time a full time assistant to the secretary has been found necessary to keep up with the increasing volume of office work.

A close-up of the filing system. There is a folder for the correspondence of and the data on every member of the Society; also ledger cards with alphabetical and geographical classification.

It is indeed a source of gratification to observe the gigantic strides that have been made by the American Society of Clinical Pathologists as reflected in the scene of busy activity in the offices of the administration.

Program of the Fourth Annual Meeting of the American Society of Clinical Pathologists

Benjamin Franklin Hotel, 9th and Chestnut Streets, Philadelphia, Pennsylvania.

WEDNESDAY, MAY 20, 9 A.M.

Call to Order—Short Business Session

Scientific Program

The Preparation of Solutions of Dextrose for Intravenous Administration, by Dr. Ralph G. Stillman, New York City, N. Y.

Utilization of Carbohydrates, by Dr. W. G. Karr, Philadelphia, Pennsylvania (by invitation).

Diagnostic Value of Spinal Fluid Sugar Content, by Dr. W. Parker Stowe, Rochester, New York.

Alveolar CO_2 Tension and Acetone in the Expired Air in Acidosis, by Dr. Paul Roth, Battle Creek, Michigan.

The Effect of Varied Carbohydrate Food in the Blood Sugar Concentration, by Dr. Leon Jonas, Philadelphia, Pa. (by invitation).

WEDNESDAY, MAY 20, 2 P.M.

Aids to Urinalysis in a Large Hospital, by Dr. C. Pons and Dr. E. B. Krumbhaar, Philadelphia, Pennsylvania (by invitation).

A New System of Duplicating and Placing Laboratory Reports in the History, by Dr. Frank W. Hartman, Detroit, Michigan.

Value of Method of Keeping Records of Tissue Examinations, by Dr. L. A. Turley, Norman, Oklahoma.

A New Type of Instrument for the Estimation of Hemoglobin, by Dr. C. E. Roderick, Battle Creek, Michigan.

Some Useful Apparatus, by Dr. Max Shaweker, Dover, Ohio.

The Organization of a Metabolism Laboratory, by Dr. William H. Stoner, Philadelphia, Pennsylvania (by invitation).

Value of the Leucocyte Fragility Test in the Prognosis of Pneumonia, by Dr. C. Pons and Dr. E. P. Ward, Philadelphia, Pennsylvania. (By invitation).

The Value of the Icteric Index in Differentiating Anemia, by Dr. A. V. St. George, and Dr. A. L. Brown, New York City.

A Plea for a Standard Method of Determining and Reporting Hemoglobin Values, by Dr. E. Clarence Rice, Washington, D. C.

Myocardial and Endocardial Changes in Cardiac Defects, by Dr. Maude E. Abbott, Philadelphia, Pennsylvania.
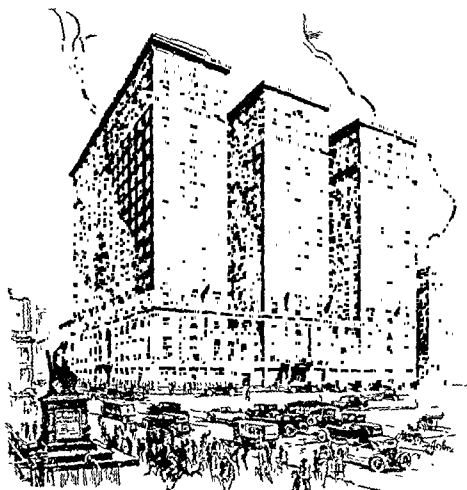
The Benjamin Franklin, Philadelphia
Headquarters, American Society of Clinical Pathologists
May 20, 21, 22, 23, 1925

THURSDAY, MAY 21, 9 A.M.

Autopsy Report of Two Cases of Thymic Death During Surgical Operations, by Dr. Jessie W. Fisher, Middletown, Connecticut.

Some Enzyme Studies with Desiccated Gonadal Tissue, by Dr. Herman Sharlit and Dr. William G. Lyle, New York City, N. Y.

A Comparison of Enzyme Actions of Tumors and Normal Tissues, by Dr. K. George Falk and Helen Miller Noyes, New York City, N. Y., (by invitation).

A Double Hydrogen Electrode System for the Determination of Hydrogen-Ion Concentration, by Dr. George H. Meeker and Mr. Bernard Over, Philadelphia, Pennsylvania, (by invitation).

Observations on the Dick Test, Toxin Immunization and Serum Treatment of Scarlet Fever, by Dr. John A. Murphy, Philadelphia, Pennsylvania (by invitation).

Pneumococcus Antibody Solution, by Dr. Frank M. Huntoon, Glenolden, Pennsylvania.

Anisocytosis and Increased Red Blood Cell Volume with Little or no Anemia, (Lantern Slides), by Dr. Mortimer Warren, Portland, Maine.

THURSDAY, MAY 21, 2 P.M.

Normal and Pathologic Basal Metabolic Rate in Obesity, by Dr. Horry M. Jones, Chicago, Illinois.

Studies on Blood Cultures with Special Reference to the "Massive" Method, by Dr. Herbert Fox and Dr. William G. Leaman, Philadelphia, Pennsylvania.

A Comparative Study of Liver Functional Tests, by Dr. A. I. Rubenstone and Dr. Louis Tuft, Philadelphia, Pennsylvania.

The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method, by Dr. B. S. Parks, Philadelphia, Pennsylvania (by invitation).

Studies on the Bacteriology of the Urine in Cooperation with Catheterization of the Ureters, by Dr. Robert A. Keilty, Danville, Pennsylvania.

Study of Cases of Acute Leukemia and Acute Mononucleosis, by Dr. B. L. Crawford, and Dr. Harold W. Jones, Philadelphia, Pennsylvania.

Blood Counts in Mississippi, by Dr. Leon S. Lippencott, Vicksburg, Mississippi.

THURSDAY, MAY 21, 7 P.M.

Reaction After Typhoid Vaccination, by Dr. Henry J. Nichols, Washington, D. C.

The Measurement of Cloudiness in Liquids, by Dr. William G. Exton, Newark, New Jersey.

Studies in Embalming Fluids in Relation to Gross and Histological Tissue Examinations, by Dr. John A. Kolmer and Dr. Fred Boerner, Philadelphia, Pennsylvania.

The Specific Inflammatory Reaction of Immunized Animals (Arthus Phenomenon), by Dr. Eugene L. Opie, Philadelphia, Pennsylvania (by invitation).

Methods of Staining Tubercle Bacilli, by Dr. H. J. Corper, Denver, Colorado.

FRIDAY, MAY 22, 9 A.M.

Sedimentation Rate of Erythrocytes, by Dr. H. N. Cooper, Watertown, New York.

Standardization of Tuberculin, by Dr. Joseph D. Aronson, Philadelphia, Pennsylvania (by invitation).

Bronchial Spirochetosis, by Dr. Thomas L. Ramsey, Toledo, Ohio.

Etiological Studies in Psoriasis, by Miss Mary Marcus, Philadelphia, Pennsylvania (by invitation).

Squamous Cell Carcinoma of the Gall Bladder, by Dr. Frank W. Hartman, Dr. W. E. Johnson, Detroit, Michigan.

The Technic of the Practical Application of the Pathogen Selective Cultural Method, by Dr. A. I. Rubenstone, Philadelphia, Pennsylvania.

FRIDAY, MAY 22, 2 P.M.

A System of Laboratory Approval Proposed to the American Society of Clinical Pathologists, by Dr. Frederick E. Sondern for the Committee on Standardization.

Discussion to be opened by Dr. Ruth Gilbert, Dr. C. Y. White (by invitation) and Dr. S. R. Haythorn.

Open Discussion

How Can We Best Promote the Objects Contained in Article II of Our Constitution? By Dr. Herman Spitz, Nashville, Tennessee.

Suggested Method to be Followed in Developing a Standardized Course for Medical Technicians, by Dr. Walter E. King, Detroit, Michigan.

FRIDAY, MAY 22, 7 P.M.

Annual Dinner in Ball Room of the Benjamin Franklin Hotel.

Presidential Address

The Functions of a Hospital, by Dr. George H. Meeker, Dean of the Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

SATURDAY, MAY 23, 9 A.M.

Business Session

SATURDAY, MAY 23, 2 P.M.

The Clinical Significance of Anticomplementary Serum, and Spinal Fluids in the Wassermann Reaction, (Kolmer Modification), by Dr. A. H. Sanford, Rochester, Minnesota.

A Standardized Wassermann Report, by Dr. A. J. Casselman, Camden, New Jersey.

The Present Status of the Kolmer Complement-Fixation Test for Syphilis as Determined by a Comparison with Numerous Other Methods, by Dr. Robert A. Kilduffe, Atlantic City, New Jersey.

Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, by Dr. Robert G. Owen, Detroit, Michigan.

Kahn's Precipitation Reaction as Compared to Kolmer's Complement-Fixation Test, by Dr. A. S. Giordano, South Bend, Indiana.

A Clinical Study of the Kolmer and Kahn Reactions in Syphilis, by Dr. R. L. Kelly, Louisville, Kentucky (by invitation).

The Meinecke Reaction as Compared with the Wassermann in One Thousand Specimens of Blood Sera. (Lantern Slides), by Dr. A. M. P. Saunders, Dunning, Illinois.

Scientific Exhibit

Microscopic and Other Specimens Relating to Tropical Medical Problems, by Dr. J. M. Feder, Santo Tomas Hospital, Panama.

Commercial Exhibits

The commercial exhibits are an added attraction to the Meeting of the American Society of Clinical Pathologists. Many of the leading laboratory houses will display a large line of new and up to the minute laboratory equipment.

Every form of apparatus will be on display in these exhibits that goes to make a complete diagnostic clinical laboratory. The leading medical publishers will exhibit a complete line of laboratory books, as well as a large number of books on allied subjects.

All of the exhibits are being planned to serve the convenience of the clinical pathologist, and it will be to his advantage to visit them often.

The Program speaks for itself and is a sufficient lure for all Clinical Pathologists to temporarily forsake their laboratories and participate in this intellectual and scientific treat.

All members should make plans immediately for attending our next meeting. The Secretary will gladly make hotel reservations for the visitors and their families. Please communicate at once with Dr. Ward Burdick, Children's Hospital, Denver, Colorado.

BOOK REVIEWS

Canned Foods in Relation to Health*

IN his Milroy lectures for 1923, published as a volume of the Cambridge Public Health series, William G. Savage undertakes an exhaustive study and criticism of the methods of preparation, the possibilities of contamination, and other potential sources of danger in connection with canned goods. Written originally as a lecture before the Royal College of Surgeons, it has an easy style which makes the volume very pleasant reading. Savage has undertaken a thorough study of canning methods both in England and in the United States and has covered the literature thoroughly. Among other points, acid foods kept in a tin for a long time will dissolve sufficiently large amounts of tin to become of pathologic significance.

That canning is not 100 per cent efficient is indicated by the observation that, in 1920, the wastage of canned meat alone was 1,354,272 pounds. A great deal of this is due to the fact that bacteria are not entirely destroyed in the process of canning. In investigations of different classes of foods by several observers no class of products was found to be always sterile, the percentage varying from 18 per cent not sterile for unsweetened milk to 84 per cent not sterile for Crustacea and 100 per cent for sweetened milk. Practically all bacteria except resistant spore bearers are destroyed. These remain viable and cause no further damage provided there is no unusual access of air.

The two most important factors in spoilage are conditions within the tin controlling the availability of free oxygen, and the initial number of organisms present. The essential importance of absolutely air-tight retainers is not that outside bacteria might gain access, but that the admission of air may render conditions suitable for the multiplication of those bacteria which are already present. Even obligate anaerobes may be stimulated to become actively decomposing organisms by the introduction of air.

Even in the absence of bacteria, that is, in properly sealed cans, with sound contents, certain chemical changes occur with aging, known to the trade as maturation. In the case of marine products this is especially desirable and improves the product. There is no evidence so far that maturation in sound tins results in the formation of any substance dangerous to health.

Recent studies of botulism and the effect of canning upon vitamines are discussed in detail.

The volume constitutes a distinct contribution to the science and the industry of canning and will be of particular value to students of public health and of industrial bacteriology.

CLINICAL AND EXPERIMENTAL

INSULIN: ITS INTRADERMAL INJECTION*

By Ernst F. Müller, M.D., and H. B. Corbitt, A.M., New York, N. Y.

IN a previous article[1] dealing with the skin and the effect of insulin on the blood sugar content observations were described showing that the results of insulin injections in rabbits vary with the route of administration. Similar amounts of insulin, taken from the same container, produce different results on the blood sugar content depending upon whether it is injected subcutaneously, or intradermally.

After subcutaneous injection of insulin, a decrease in the blood sugar content was noted between the fifteenth and forty-fifth minute. The lowest figure obtained with 0.5 and 1 unit of insulin per kilo body weight in accordance with other observers, was about 50 per cent lower than the initial findings, but it rarely dropped to less than 0.45 mg. per cubic centimeter. This low level was maintained during the first two hours. Four hours after injection the decrease did not exceed 25 per cent of the original status and after six hours the initial figures of the blood sugar contents again prevailed. Similar curves were found to obtain in almost all of the cases examined where the drug had been administered subcutaneously, but a fundamental difference in the curve of the blood sugar content was observed after intradermal injection of 1 unit and of 0.5 unit of insulin. The sugar decrease began about the same time as after subcutaneous injection. The lowest point of the curve was also reached after a lapse of time corresponding to that in the foregoing tests. The striking difference became evident on comparison of the data obtained four hours after the intradermal injection. While the subcutaneously injected animals showed a decrease of about 25 per cent below normal at this time, the low blood sugar content noted

after two hours in both kinds of animal tests was still observed in those injected intradermally. In other words the rabbits injected intradermally had a more prolonged blood sugar reaction than those treated subcutaneously with the same agent. Similar differences were demonstrated when determining the blood sugar content after a six hour period. At this time almost all the subcutaneously injected animals reached the normal level while the others still showed a decrease of about 25 per cent below normal.

Heretofore, there has been no explanation of the fact that insulin acts differently according to the route of administration. A detailed discussion of our findings and conclusions will be found in our previous articles. The question now arises as to the importance of the time of resorption. According to the findings mentioned above, the time of resorption, different though it may be in the skin and in the subcutaneous tissues, is not the cause of the difference in the blood sugar curves. The fact that, after both modes of administration, the lowest point of the curve was reached at almost the same time, contradicts any explanation by the resorption theory alone. If the specific substances reach the blood stream only through a slow process of resorption within the skin, then surely the onset of the reaction must be delayed. This not being the case the resorption theory cannot be accepted as an adequate explanation of this phenomenon.

Therefore it is concluded that the skin, or a special function of the skin, possesses the power of affecting the insulin reaction in the body in a way hitherto unknown. The skin as a special organ is closely related to the involuntary nervous system, as the body is related to the blood cells and to the serum, and is, therefore, capable of producing certain reactions in the body. It is known, for instance, that 0.1 c.c. of aolan, a nonspecific lactalbumin preparation, germ-free, toxin-free, and nonirritating, is an effective stimulant of the involuntary nervous system if given intradermally; on the other hand, subcutaneous, intramuscular or even intravenous administration of the same quantity of this agent is not followed by any measurable change in the organs controlled by the involuntary nervous system. These facts, briefly outlined on account of lack of space, make it seem highly probable that the effect of the intradermal administration on the insulin reaction is due to a special property of the skin, as yet unknown. If, as our tests show, the injection of a specific agent (insulin), intradermally, increases the effect of such agent as compared with its subcutaneous or intravenous employment, it must be concluded that an added feature of skin physiology has been discovered. Because of the great importance of this problem, as outlined above, it is desired to confirm and establish previous results. New studies along these lines have accomplished this, and it is our desire to present additional data of a confirmatory nature, thus providing an impetus to still further experimental work.

In our former experiments two animals were used for each test and the results obtained by intradermal and subcutaneous injection were carefully compared. It is known that animals, particularly rabbits do not respond uniformly to equal amounts of a given agent. The importance of this has not been given due consideration. But now the observation, that the response of the animal

organism varies with the route of administration, takes on a new value since it may be essential to establish the relationship between the skin and the involuntary nervous system on the one hand and the blood sugar content on the other; furthermore, it may be a factor in recognizing the rôle of the skin in the purely specific processes mentioned in a recent paper.[2]

In an article by Macleod and Orr[3] it is stated that rabbits, on the same diet and starved for twenty-four hours preceding the tests, cannot be considered strictly uniform since they vary in sex, age, and breed, and they also vary considerably in their susceptibility towards insulin, both with regard to effect on blood sugar and the incidence of convulsions. These variations have been recognized by us. It is our desire to present in detail the experiments whereby these factors have been controlled.

In their article Macleod and Orr have also given illuminating explanations of these effects which have been mentioned briefly by us. It is stated that the following must be taken into consideration: (1) Variability in the carbohydrate metabolism of different animals; (2) seasonable variations; (3) the development of an immunity to insulin.

As stated above, every possibility of such errors has been eliminated, as far as such elimination in biologic tests is possible. In the first place, variability in the carbohydrate metabolism of different animals as well as other individual differences between two animals, may be considered as nonexistent by using the same animals for the different tests after a week or two of rest. By conducting our experiments in this way, it was expected to avoid the other sources of error mentioned by Macleod and Orr in the second and third parts of their conclusions.

An interval of not more than two weeks between the tests in a single animal as well as the examination of the entire series within one month, may be considered a sufficient precaution to avoid mistakes by seasonal variations. The well-established statement by Macleod concerning the development of an immunity to insulin can evidently be ignored in *our* tests, because the same animals were not used more frequently than three or four times at the most.

Also by comparing these later tests of thrice used rabbits with the first tests in other animals, not previously used for insulin injections, there was no manifestation of an immunity to insulin in the less frequently used rabbits.

In Table I the results of experiments on seven different rabbits are given. Three of these animals were injected twice subcutaneously and twice intradermally; one was injected twice intradermally and once subcutaneously; the other three had one injection each by the subcutaneous and by the intradermal route. In the first column are recorded the results of the intradermal injections as shown by the blood sugar content in comparison with the initial blood sugar content. In the second column are found the corresponding values for subcutaneous injection. Each horizontal line shows two tests on the same animal by different methods of injection. Thus comparison may be made in the same animal, of the effects of the different routes of administration, and between different animals using the same method of administration.

Table II gives the same results by tabulating the percentage increase in the blood sugar content, only. These values are tabulated in the same manner as in Table I, showing in the first column the findings after subcutaneous insulin injections. The subdivisions in this table give the decrease in percentage in the second, fourth, and sixth hours as compared with the initial blood sugar content of each individual animal. In view of the fact that both tables deal with the same tests and that both are submitted only to facilitate a complete review of the data, our discussion of the findings will refer only to the second table. A graphic presentation of these results is found in Chart 1.[4]

TABLE I

SUMMARY OF EXPERIMENTS ON MODE OF INJECTION
THE BLOOD SUGAR CONTENT AFTER THE INJECTION OF INSULIN

	BLOOD SUGAR: MG. PER 100 C.C. HOURS AFTER INTRADERMAL INJECTION				BLOOD SUGAR: MG. PER 100 C.C. HOURS AFTER SUBCUTANEOUS INJECTION			
RABBIT NO.	0	2	4	6	0	2	4	6
58	152	62.5	63.5	47	123	45	100	129
58	115	39	49	67				
78	155	70	96	134	134	66	102	123
78	130	73	90	117	139	52	120	131
79	156	91	96	118	130	77	81	99
80	123	56	89	136	129	63	117	125
80	121	52	91	126	131	45	131	132
81	117	34	34	59	115	46.5	67	103
82	142	96	117	127	127	98	128	134
83	129	76	88	111	128	83	118	114
83	122	61.5	64.5	72	121	62.5	116	115
Average	132.9	64.6	79.9	101.3	127.7	63.8	108	120.5

TABLE II

SUMMARY OF EXPERIMENTS ON MODE OF INJECTION
PERCENTAGE CHANGE IN SUGAR CONTENT

RABBIT NO.	HOURS AFTER INTRADERMAL INJECTION			HOURS AFTER SUBCUTANEOUS INJECTION		
	2	4	6	2	4	6
58	−58.5	−58.0	−69.0	−63.5	−18.7	+ 4.9
58	−66.1	−57.4	−41.8			
78	−54.8	−38.3	−15.7	−50.8	−23.9	− 8.2
78	−43.8	−30.8	−10.0	−62.6	−13.7	− 5.8
79	−41.7	−38.5	−24.3	−40.8	−37.7	−23.8
80	−51.2	−27.6	+10.6	−51.2	− 9.3	− 3.1
80	−57.0	−24.8	− 4.1	−65.7	0.0	+ 0.8
81	−71.0	−71.0	−49.6	−59.7	−40.8	−10.4
82	−32.4	−17.6	−10.6	−22.8	+ 0.9	+ 5.5
83	−41.2	−31.0	−14.0	−35.2	− 7.8	−10.9
83	−49.6	−47.2	−41.0	−48.4	− 4.1	− 5.0
Average	−51.57	−40.2	−23.75	−50.07	−15.52	− 5.5

Table II.—Most of the findings are sufficiently clear to render needless an explanation of all the tests in detail. It is desirable, however, to examine the following individual tests on Rabbits 58, 81, 82 and 79. Rabbits 58 and 81 are both sensitive to the action of insulin. In Rabbit 81 convulsions ensued from an intradermal injection of 0.5 unit of insulin. In both animals the blood sugar level was quite low at the four and six hour bleedings following intradermal injection. Yet after the subcutaneous injection of the same amount of insulin

both recovered rapidly and were nearly normal at the end of the sixth hour. Rabbit 82, on the other hand, was not so sensitive to the action of this drug when given in the same amount and of the same lot number. This lack of reactivity was even more evident by the subcutaneous route of administration.
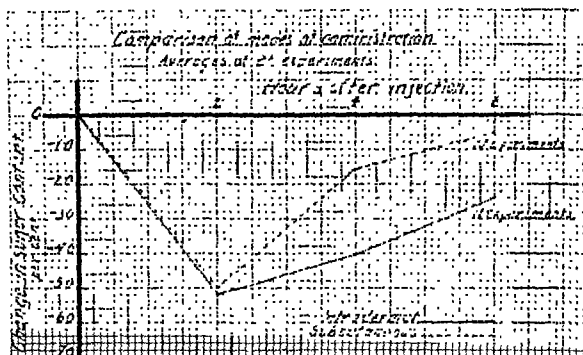
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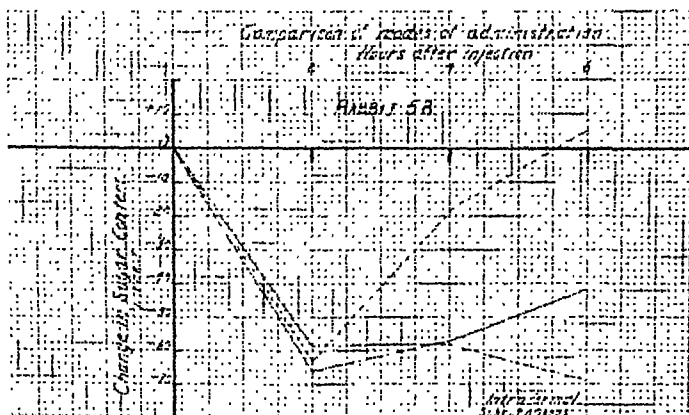
Chart 2

Perhaps it should be noted here, that in other experiments to be published elsewhere, this rabbit was hypersensitive to the action of suprarenin and its derivatives. Still another type of animal is represented by Rabbit 79, little difference being observed between the results of the two methods of injection. Only one

animal of this type has been observed. A graphic illustration of these results is shown in Charts 3 to 5.

Chart 1 to 5.—Chart 1 gives a graphic presentation of the average changes in the blood sugar content after intradermal and subcutaneous injections, ob-
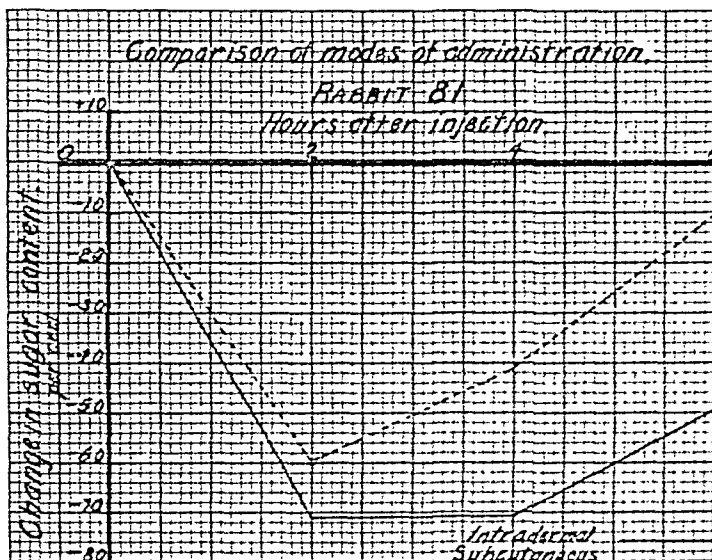
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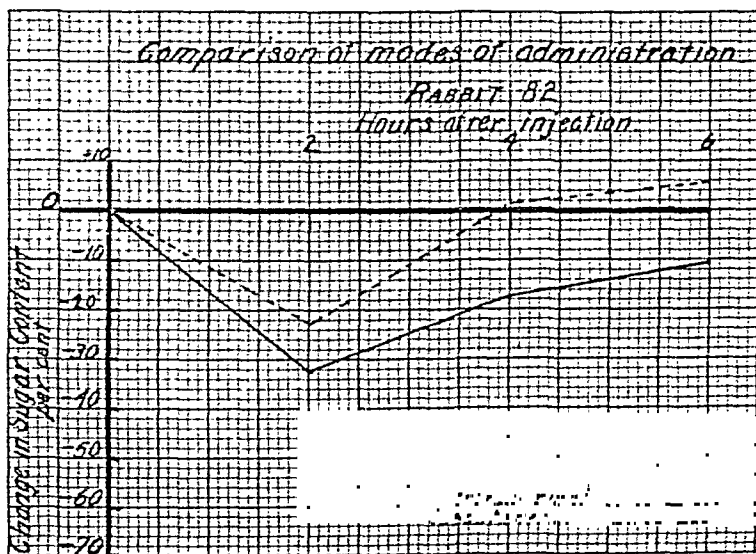
Chart 4.

tained from the above twenty-one tests in these seven animals and the variations from this average. These results may be regarded as additional proof of our previous findings and the variations of the effect of insulin.

Before summarizing the above findings, one more point must be mentioned. The amount of consideration which should be given to the effects of struggling

and its influence upon the results of experiments of this nature, has been shown in another paper,[4] viz.: properly controlled experiments are not affected by struggling as far as their results are concerned with the effect of insulin.

It must be concluded that after intradermal administration there is evident a prolongation of the effect of insulin on the blood sugar level of normal rabbits. These results are all the more important as they are obtained in accordance with
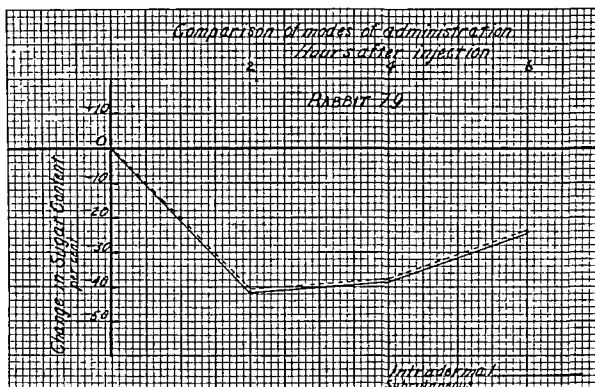
Chart 5.
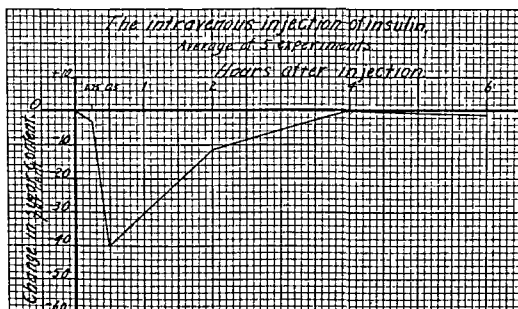
Chart 6

the precautions prescribed by Macleod and Orr. Without going into further details, there is only one explanation possible, namely, that the skin as an individual organ is partly responsible for this effect. Of course while the factor of resorption plays an important part, it alone does not suffice to explain the phenomenon, since resorption constitutes only a special property of the skin which may influence the effect by its physiologic action. The following was

taken into consideration: the effect of insulin injections increases proportionately to the distance from the circulatory system at which the injection is made. It was, therefore, necessary to include a comparison of the effect of intravenous injection on the blood sugar content. These findings are given in detail in the paper mentioned above and show that the effect of insulin was not very marked when injected directly into the blood stream. The results following intravenous injection as given in Table III and Chart 6 show a decided difference as compared with previous figures. (As regards Chart 6, attention is directed to the fact that each single point of the curve represents a mean of the individual findings and that this average was not always obtained from an equal number

TABLE III

THE EFFECT OF INTRAVENOUS INJECTION OF 0.5 UNITS (PER KG. BODY WEIGHT) OF INSULIN
UPON THE BLOOD SUGAR OF NORMAL RABBITS

RABBIT		UNIT PER KG.	TOTAL UNITS INJECTED	TIME AFTER INJECTIONS (HOURS)	BLOOD SUGAR CONTENT (MG.PER 100 C.C.)	CHANGE IN SUGAR CONTENT PER CENT
NO.	WT.					
80	2060	0.5	1.0	0.0	137	
				0.5	96	−40.0
				1.0	107	−21.9
				2.0	132	− 3.7
				4.0	138	+ 0.7
				6.0	138	+ 0.7
83	2450	0.5	1.2	0	121	
				1	76	−37.2
				2	97	−19.85
				4	123	+ 1.6
				6	122	+ 0.8
80	2075	0.5	1.0	0	138	
				2	113	−18.1
				4	126	− 5.8
				6	123	−10.9
81	2150	0.5	1.1	0.0	120	
				0.25	124	+ 3.3
				2.00	91	−24.2
				4.00	125	+ 4.2
				6.00	127	+ 5.8
82	2250	0.5	1.1	0.0	129	
				0.25	117	− 9.3
				2	141	+ 9.3
				4	129	0.0
				6	127	− 1.6

of animals. The number of determinations represented by every point of the curve is readily seen in Table III.) One-half hour and one hour after intravenous injection there was a marked decrease in the blood sugar content; however, this level is but little below the initial findings and lasted only for a short time. At the end of the two hour period the blood sugar decrease had greatly diminished and corresponds to the four hour level after subcutaneous, and to the six hour level after intradermal injections. Four hours after intravenous injection the blood sugar content was almost normal. Perhaps the most important of these findings is that the blood sugar decrease thirty minutes after injection was comparatively small. Therefore, insulin, which enters the blood stream in highly concentrated form, is not able to produce a decrease in the blood sugar

content in proportion to this higher concentration. This observation is sufficient proof that the factor of resorption alone does not explain our findings. We have thus by tests of intravenous administration supported our former conclusion that the effect of insulin injections increases proportionately to the distance from the circulatory system at which the injection is given. In other words, intravenous injections are the least effective, subcutaneous injections are considerably more so and the intradermal mode of administration is by far the most effective. These findings suggest a comparison with earlier experiences with nonspecific agents. Their effect, especially when nontoxic and nontissue-irritating preparations are used, is also greatly enhanced by the intradermal method of injection; while equal doses administered intravenously are ineffective, and it has been definitely determined that this increased effectiveness is due to the close relation of the skin to the involuntary nervous system.

It was our aim to exclude certain sources of error cited by Macleod and Orr as also certain factors which might influence the final results, viz.: struggling of the animals. Furthermore, it was our intention to broaden the basis of our investigations by conducting the same tests in the same animals thus basing our knowledge upon results secured with identical biologic conditions. A special series of experiments, differing in the method of attack from those in this paper, will be necessary to carry on these studies; the foundations for which have been laid by this and previous papers.

CONCLUSIONS

1. The effect of insulin varies with the route of administration. The effect of insulin injections upon the blood sugar content of normal rabbits is markedly increased if the intradermal method of administration is employed; subcutaneous injections of the same amount of insulin into the same as well as into different animals are less effective.

2. A comparison of intradermal, subcutaneous, and intravenous injections of equal amounts of insulin shows that their effectiveness decreases in the order mentioned.

3. The degree and the period of effectiveness does not depend upon the rapidity of resorption, but upon a hitherto unknown factor, very probably related to the action of the involuntary nervous system.

4. This property seems to be inherent in the skin rather than in the subcutaneous tissues or in the body fluids.

REFERENCES

[1]Müller, E. F., and Corbitt, H. B.: JOUR. LAB. AND CLIN. MED., 1924, ix, 608.
[2]Müller, E. F.: Arch. Int. Med. (to be published soon).
[3]Macleod, J. J. R., and Orr, M.D.: JOUR. LAB. AND CLIN. MED., 1924, ix, 591.
[4]Corbitt, H. B.: Jour. Am. Pharm. Assn., 1925, xiv, 108.

with the blood filtrate (about 3.4 c.c. in normal infants) is subtracted from the amount used with the blank (about 3.9 c.c.). The value obtained is multiplied by five which gives a figure which corresponds to that which would have been obtained by the original Shaffer-Hartmann method. From their table the corresponding blood sugar values are obtained for all concentrations of 0.1 per cent or over. Below this concentration it was found that a definite but constant error occurred when their table was used. This necessitated the construction of a new table for values below 0.1 per cent. These figures are given in Table I.

The values found on known theoretical solutions of dextrose are given in Table II and a comparison of the amount of blood sugar found by the standard Schaffer-Hartmann method and the modified method are given in Table III.

It is seen that the procedure here used, consists of the Schaffer-Hartmann method in its entirety, only in the first stage one-tenth of the amount of blood
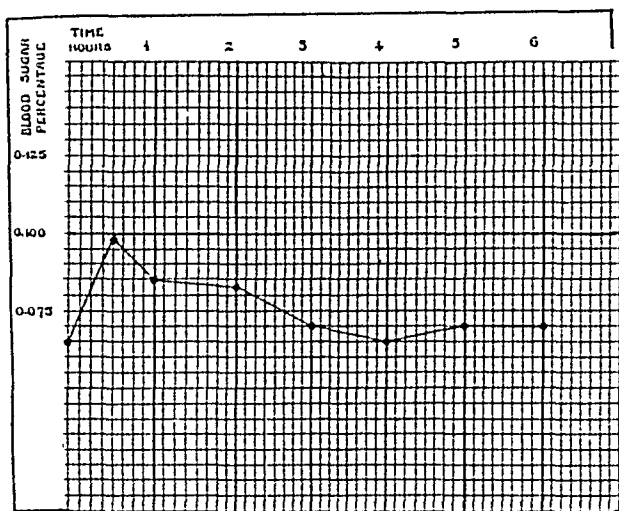
Chart 1.—Baby D. K., weight 8 pounds, 12 ounces. Blood sugar curve after feeding 4 ounces of the mixture of lactic acid, milk 10 ounces, water 10 ounces, and corn syrup 1 ounce.

is used, while in the latter stage one-fifth the amount of filtrate is used and the final titration multiplied by five.

The average blood sugar found in twenty approximately normal infants was 0.090 per cent. The lowest value found was 0.081 per cent and the highest 0.100 per cent. These infants were not perfectly normal but as nearly normal as can be obtained in an active service of a children's hospital. The blood sugar was determined in twelve cases of marasmus. These infants were all only two-thirds to one-half of their normal weight for their age. The average value found was 0.077 per cent. Only two of the infants had blood sugar values below 0.060 per cent.

The time in which the blood sugar returned to its usual level after an average feeding was studied in six cases. They were all infants suffering with marasmus. Two infants were gaining in weight, two were losing and two were stationary in weight. The same type of curve was found whether the infant was gaining or losing The average initial blood sugar content was 0.070 per

cent. The average maximum height reached was 0.102 per cent, which is an increase of 46 per cent over the initial value. The maximum concentration of blood sugar was reached in half an hour in four cases and in one hour in two cases. At the end of two hours the values were usually slightly above the original figures, while at three hours they were approximately the same as those obtained at the initial determination. The blood sugar then remained nearly stationary for the next three hours with a slight tendency to rise. No
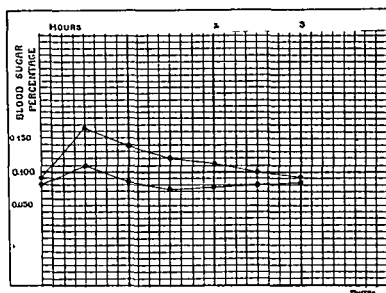
Chart 2.—The lower line represents the curve of the average blood sugar values found after the interstitial injection of five per cent glucose. The upper line represents the curve of the average blood sugar values found after the interstitial injection of 10 per cent glucose. The infants were approximately normal.
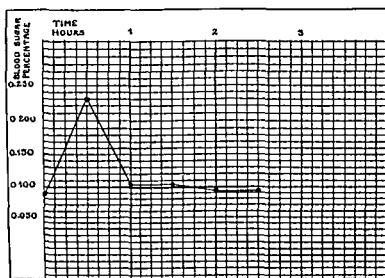
Chart 3.—Illustrates the rapidity with which the blood sugar may drop in certain normal infants after the interstitial injection of 10 per cent glucose.

determinations were made more than six hours after the feeding. The feedings consisted of lactic acid milk ten ounces, water ten ounces, and corn syrup one ounce. The carbohydrate content was 8.5 per cent and the amount given was 0.4 ounces per pound of body weight. The curve of values found with one infant is shown in Chart 1.

Blood sugar determinations were made after the oral administration of one ounce of the various carbohydrates in common use. Entirely different curves were obtained with different carbohydrates but as the results were so variable we do not feel justified in presenting them at this time.

The blood sugar curves, after the administration of glucose interstitially, were studied in sixteen cases. Three infants received 5 per cent glucose, eleven 10 per cent and two 15 per cent. A preliminary fasting blood sugar estimation was made, followed by half-hourly determinations for three hours after the conclusion of the injection. In most instances it required from fifteen to thirty minutes to give the interstitial injections, although occasionally it required as much time as forty-five minutes. Approximately 10 c.c. of solution per pound of body weight was given. With the five per cent glucose the initial value in three cases was 0.078 per cent. The highest value was obtained on the aver age in half an hour after all the glucose was given. The average value was 0.110 per cent, a percentage increase of forty. The surprising observation was made that the blood sugar level returned to the original value in an hour in two cases, and an hour and a half in the third case. In Chart 2 is a plotted curve of the average values obtained.
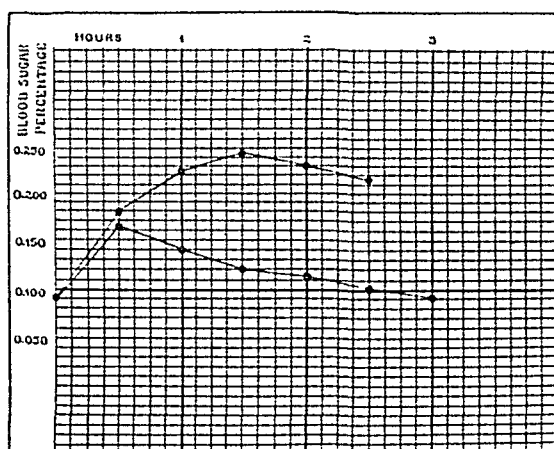
Chart 4 —The upper line represents the curve of the blood sugar values found after the interstitial injection of 10 per cent glucose in an infant suffering from acute intestinal intoxication. For comparison the lower line gives the average values found in normal infants after the interstitial injection of 10 per cent glucose.

Ten per cent glucose was given interstitially in eleven cases; seven of these infants were approximately normal. The average initial value in these seven cases was 0.090 per cent. The highest value was obtained on an average in slightly over half an hour after all the glucose was given. The average of the highest values was 0.165 per cent. The time required to return to the original figure varied from one hour to three hours. In Chart 2 is a plotted curve of the average values obtained with the seven approximately normal infants. In some of these cases the drop in blood sugar concentration after the interstitial occurred very rapidly. This is illustrated in Chart 3.

In the four abnormal cases the blood sugar curve dropped more slowly. These infants suffered from acute intestinal intoxication, lobar pneumonia, tetany with nasopharyngitis, and Potts' disease. Attention is directed only to the results obtained with the infant which suffered from intestinal intoxication, as the values with the other cases more nearly approached the normal figures. In Chart 4 is plotted the curve obtained in this case with the average values

of the seven normal cases for comparison. Reference will be made to this case later.

In regard to the use of fifteen per cent glucose, interstitially, the values found in two normal infants did not differ materially from those found with the use of the ten per cent solution.

Glucose was given intravenously to ten infants. Five of the infants were approximately normal while the other five were in poor condition. The pathologic conditions present in the infants in the latter group were fermentative diarrhea and mild intestinal intoxication. A ten per cent solution of glucose was employed and the infants were all given approximately 10 c.c. per pound of body weight. In Chart 5 is shown the average curves of the blood sugar content of both the normal and abnormal infants. It is to be noted that the blood sugar in the five normal infants returned to
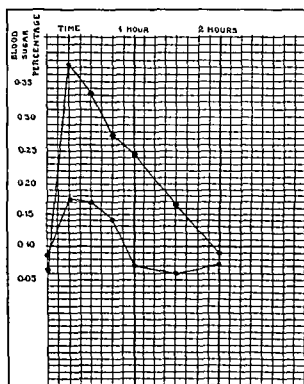
Chart 5.—The upper line represents the curve of the average blood sugar values obtained after the intravenous injection of 10 per cent glucose to five infants suffering from acute intestinal intoxication and fermentative diarrhea. The lower line represents the curve of the average blood sugar values obtained after the intravenous injection of 10 per cent glucose to five approximately normal infants

the original level in one hour's time; in fact the value was actually below the initial value (initial value 0.082 per cent, one hour later 0.071 per cent). The maximum height of the curve was 0.174 per cent. In contrast to the normal infants the blood sugar concentration in the five abnormal cases did not return to the original level within two hours. The height of the curve was almost double that obtained with the normal infants and at one hour after the injection, the average concentration was 0.247 per cent instead of less than 0.100 per cent, as was found with the first group of infants. One and a half hours after the injection the concentration was still 0.167 per cent.

It appears that infants acutely ill with the above pathologic conditions are not able to utilize the glucose given either intravenously or interstitially with the same rapidity as the normal infant. This of course at once brings up the

question, is there a deficiency of pancreatic function in these cases? The most constant pathologic finding in this type of case, however, is a large fatty liver. This condition is also found in cases of intoxication following anesthesia and it is known that this can frequently be prevented by the preliminary administration of carbohydrate. It appears that when glycogen is deposited in the liver it protects the organism from the effects of an intoxication. The question arises, will glycogen be deposited in the livers of these infants if insulin is given with the glucose? This is not at all certain. Professor Macleod[3] has found, that in the case of a normal animal, the administration of insulin and glucose is not followed by the deposition of glycogen in the liver, but such deposition does occur in a diabetic animal.

At the present time we are continuing our studies on this most interesting aspect of carbohydrate metabolism. We hope that some valuable information
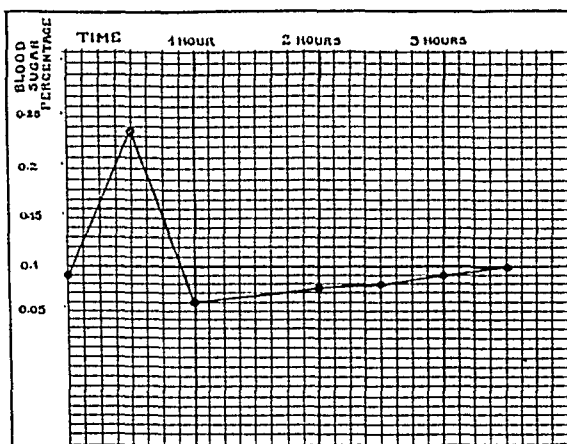
Chart 6.—Blood sugar curve obtained in Baby E. S. after the intravenous injection of 10 c.c. per pound of body weight of 10 per cent glucose. It is to be noted that at the end of one hour the blood sugar concentration is less than before the glucose was introduced. The blood sugar concentration then slowly increased. This curve might be taken to indicate an overproduction of insulin under the stimulus of the injected glucose.

may be obtained by including in this work the determination of the respiratory quotient, and also the glycogen and fat content of the liver of any suitable cases coming to autopsy.

SUMMARY

A procedure has been devised whereby blood sugar determinations may be made with the use of only 0.2 c.c. of blood. The procedure is based on the Shaffer-Hartmann method. The results obtained are usually accurate within 5 per cent.

The average fasting concentration of blood sugar in twenty approximately normal infants was found to be 0.087 per cent. In twelve cases of marasmus, the average concentration was 0.070 per cent. Values as low as 0.050 per cent were obtained in the latter group.

When 5, 10, and 15 per cent solutions of glucose are given interstitially to normal infants the blood sugar curve reaches its height about half an hour after

the injection is given. This rapidity of absorption is of practical value when an intravenous injection cannot be given readily.

The rise in blood sugar concentration in normal infants is more marked after the interstitial injection of 10 per cent glucose than after the interstitial injection of 5 per cent glucose. The fall in the blood sugar curve after interstitial injections of glucose occurs with remarkable rapidity.

The intravenous injection in normal infants of large amounts of glucose; i. e., one gram of glucose per pound of body weight, is followed by a rapid diminution of the blood sugar concentration, after the preliminary rise. One hour after injection, the concentration may be actually less than that found before the injection.

Infants with conditions such as acute intestinal intoxication and fermentative diarrhea are unable to utilize glucose introduced parenterally with the same rapidity as normal infants. A study of the effect of insulin in these cases is in progress.

Attention is again directed to the tremendous ability of the normal infant to utilize glucose introduced parenterally.

REFERENCES

[1]Tisdall, F. F., and Brown, A.: Am. Jour. Dis. Child., 1924, xxvii, 312.
[2]Shaffer, P. A., and Hartmann, A. F.: Jour. Biol. Chem., 1921, xlv, 365.
[3]Macleod, J. J. R.: Personal communication.

DIETETIC MANAGEMENT OF CARDIAC, VASCULAR, AND RENAL DISEASE*

BY JOSEPH R. DARNALL, M.D., CAPT., MEDICAL CORPS, U. S. ARMY

PROPER dietetic care of the cardiac, vascular, and renal invalid is largely dependent on the diagnostic and therapeutic judgment of the attending physician, and the diets required may vary widely for different patients. Numerous factors contribute to the undesirability of arbitrarily fitting certain type diets to certain type diseases. These factors include a careful consideration of the functional capacities of the digestive organs, heart, vessels, and kidneys, which vary in different patients, and may even vary greatly in the same patient within short periods of time. Individual likes and dislikes, idiosyncrasy, and tolerance for certain foods must be considered.

Many functional cardiac disorders, as well as mild, well compensated valvular disease, may require no dietary regulation. In cases, however, where the circulation is maintained only by excessive cardiac effort, as in chronic hypertension, and in long standing or extensive valvular disease, an effort should be made through diet, to lessen the cardiac burden. This dietary regulation is even more important, in cases of actual myocardial degeneration.

ORGANIC HEART DISEASE

The processes of digestion, assimilation, and excretion demand considerable energy, and if not properly controlled, may add to the burdens of a failing heart. Passive congestion of the digestive organs necessitates a careful selection and limitation of food and drink. In general, foods most readily digested, and producing waste products most readily excreted, should be chosen. The following rules, then, should be observed in regulating the diet in organic heart disease:

1. The diet should be relatively dry, and easily digestible.

2. Foods causing flatulence should be avoided. These foods will vary in different individuals, but in general, beans, peas, cabbage, and starchy foods are more liable to produce flatulence.

3. The heaviest meals should be at noon, and breakfast. Supper should be light. Meals should be about five hours apart, and no solid food taken between meals.

4. Fluids should be restricted to 1500 c.c. if edema is absent. In the presence of slight edema, fluids should be limited to 1000 c.c. If edema is marked, with effusion into the serous cavities, fluids should not exceed 800 c.c. If the edema persists or increases, the Karell diet may be tried. This consists of giv-

ing 200 c.c. of skimmed milk at 8 A.M., 12 NOON, 4 P.M., and 8 P.M., for from five to seven days. The diet is then gradually increased, adding first toast, then vegetables, one or two eggs, and finally a little meat.

5. Salt should be restricted, as it increases desire for liquids, and also withdraws fluid from the tissues into the blood stream, thus increasing the volume of the blood, and adding to the cardiac burden.

VASCULAR HYPERTENSION

The following dietary instructions are given to patients with vascular hypertension. Adherence to this regime is beneficial for all individuals past fifty years of age, whether or not they are suffering from increased vascular tension:

1. Restrict fluids moderately, taking about 1500 c.c. daily. Drink a glass of hot water just before breakfast, to cleanse the stomach and upper bowel and stimulate peristalsis.

2. Limit coffee or tea to one cup daily, preferably at breakfast.

3. Avoid overeating. A general quantitative reduction in diet is important. If a general reduction in diet aggravates or induces constipation, harmless bulk in the form of bran, fruits, and green vegetables may be added.

4. Avoid salty foods, such as ham, bacon, and salt fish. Do not put salt on food.

5. Eat meat (fish, flesh, or fowl), only once a day, and then only a small portion.

6. Do not eat more than one egg a day.

7. Avoid all highly seasoned foods, spiced foods, and restrict sweets.

8. Diet should be composed largely of green vegetables, potatoes, fruits, cereals, rice, hominy, spaghetti, bread (especially whole wheat and graham), and limited deserts.

Myocardial insufficiency, or impending decompensation will necessitate modification of the diet, along the lines indicated for heart disease.

CHRONIC INTERSTITIAL NEPHRITIS

In these cases, delicate judgment may be required to determine the fluid volume to be allowed. Too much fluid will burden the heart, and cause strain on damaged vessels; while too little fluid will impair the dilution and excretion of toxins through the kidney. In this disease the kidneys are unable to excrete concentrated urine.

The fluid should average between 1500 c.c. and 2000 c.c. daily, if there is not excessive hypertension,—that is, if the systolic pressure is below 200 mm. Protein should be restricted to 50-80 gm daily, and salt should not exceed 5 gm. The total calories should be limited to 2000-2500 daily. The diet, then, should be reasonably balanced, easily digestible, with moderate protein and salt restriction, and a general quantitative limitation. It is most important to preserve heart muscle nutrition and compensation, and any modification of the diet will depend largely on the efficiency of the myocardium.

CHRONIC PARENCHYMATOUS NEPHRITIS

A normal fluid intake is desirable, if there is no edema. If edema is present, allow only 1000 c.c. fluid daily, and decrease or increase, as the variation in edema indicates. Forcing fluids in these cases sometimes promotes diuresis and disappearance of the edema, apparently due to the fact that the kidney can excrete salts in dilute solution, but cannot eliminate concentrated salt solutions. It is believed that forcing fluids should not be tried unless restriction of fluids fails to bring about a definite subsidence of the edema. Protein should be limited to 50-80 gm. daily. Salt should be markedly restricted, and obstinate cases of edema may require a strict salt-free diet, no salt being used even in the cooking of the practically salt-free foods. (Occasionally restriction of salt increases the edema, and 5-10 gm. of salt in such cases may act as a diuretic.)

Epstein advanced a theory that certain cases of edema with albuminuria differ from the usual picture of chronic nephritis, and that in these cases the edema is due to depletion of albumin, and he, therefore, advocates a high protein diet. These cases suitable for high protein diet are said to present an alteration of the normal ratio of albumin to globulin in the blood, and a high blood cholesterol. He advocates 120-240 gm. of protein, 20-40 gm. of unavoidable fat, and 150-300 gm. of carbohydrate. Consensus of conservative opinion is that cases responding favorably to such treatment must be extremely rare, and that while in some cases disappearance of edema may indicate improvement, the blood nitrogen will be increased, and the phthalein excretion diminished, as has been demonstrated in cases checked with blood nitrogen determinations and phthalein excretion tests.

ACUTE NEPHRITIS

In general, the diet should be adequate for the needs of the patient, and yet demand as little excretory function as possible, on the part of the kidney. The following general rules should be observed:

1. Marked restriction of protein and salt, and moderate restriction of fluids. The protein should be reduced to 20-30 gm. and the salt to 2-3 gm. Marked edema may call for strict salt-free diet. Fluids should be reduced to 1000-1500 c.c.

2. A milk diet may be given, or if greater reduction of protein and salt is deemed advisable, a diet of fruit juices, thin gruels, crackers, toast, rice, and potatoes may be substituted.

3. If much fluid is lost by vomiting, diarrhea, or sweating, it must be replaced, to dilute the toxins and facilitate the excretion of waste products.

4. After the acute symptoms have subsided, the diet may be increased, depending for selection of foods, on the presence and relative severity of nitrogen or salt retention.

CONCLUSION

No attempt has been made to give detailed advice or set dogmatic rules, but only to present general plans for the logical dietary treatment for cases of cardio-circulatory impairment and renal insufficiency. It has been the author's inten-

tion to outline certain dietary principles which may serve as a flexible guide to the dietary management of these closely associated and often overlapping pathologic conditions.

REFERENCES

Alsever, W. D.: Treatment of Hypertension, New York State Jour. Med., August, 1921.

Barry, R. K.: Myocardial Insufficiency, Calif. State Jour. Med., October, 1922.

Bergoignan, P.: Diet in Renal and Circulatory Disease, Practitioner, August, 1919.

Blumgarten, A. S.: Rational Treatment of Chronic Nephritis, New York Med. Jour., August 24, 1918.

Christian, H. A.: Treatment of Chronic Nephritis, Jour. Am. Med. Assn., June 12, 1920.

Christian, H. A.: Brights Disease, South. Med. Jour., August, 1920.

Cross, J. G.: Causes and Care of Arterial Hypertension, Journal Lancet, October 15, 1922.

Elliott, A. R.: Essential Hypertension and Chronic Nephritis, Jour. Am. Med. Assn., May 28, 1921.

Falsh, E. F.: A Nephritic Diet, Am. Jour. Med. Sc., June, 1920.

Fontaine, B. W.: The Causes and Treatment of High Blood Pressure, South. Med. Jour., December, 1922.

Foster, N. B.: Treatment of Hypertension, Jour. Am. Med. Assn., September 30, 1922.

Friedenwald and Rurah: Textbook. Diet in Health and Disease.

Greer, A. E.: Treatment of Cardiac Decompensation, Texas State Jour. Med., February, 1922.

Guggenheimer, H.: Causal and Symptomatic Treatment of Dropsy, Berl., Klin. Wchnschr., September 16, 1922.

Houghton, H. A.: The Treatment of Arterial Hypertension with Low Sodium Chloride Dietary, Med. Rec., March 18, 1922.

Howard, Tasker: Clinical Types of Nephritis, New York Med. Jour., August 24, 1918.

Jenkins, W. A.: Myocarditis and its Management, Jour. Am. Med. Assn., June 24, 1922.

Mackenzie, J.: Textbook. Diseases of the Heart.

Mosenthal, Herman O.: The Treatment of High Blood Pressure, Med. Clin. N. Amer., January, 1922.

Newburg, L. H., and Clarkson, S.: Production of Arteriosclerosis in Rabbits by Diets Rich in Animal Proteins, Jour. Am. Med. Assn., September 30, 1922.

Newburg, L. H., and Squier, T. L.: High Protein Diets and Arteriosclerosis in Rabbits, Arch. Int. Med., July, 1920.

Osler, W., and McCrae, T.: Textbook. Practice of Medicine.

Reilly, T. F.: Digestive Complaints of the Cardiac Patient, New York State Jour. Med., December, 1922.

Ritchie, H. J.: Some Aspects of Vascular Hypertension, Med. Jour. of Australia, March 25, 1922.

Rose, R. H.: Weight Reduction and its Remarkable Effect on High Blood Pressure, New York Med. Jour., June 21, 1922.

Stone, C. T.: The Treatment of Hypertension, Texas State Jour. Med., February, 1922.

Truceneck, C.: Spasmodic and Plethoric Varieties of Vascular Hypertension, Riforma med. Naples, June 5, 1922.

Warfield, L. M.: Treatment of Chronic Nephritis, Jour. Mich. State Med. Soc., September, 1924.

White, P. D.: The Failing Heart, Boston Med. and Surg. Jour., November 23, 1922.

Young, G. S.: The Prophylaxis and Treatment of Arteriosclerosis, Can. Jour. Med. and Surg., Toronto, July, 1922.

STUDIES ON THE PNEUMOCOCCOLYTIC ACTIVITY OF THE BILE FROM EXPERIMENTAL BACTERIAL CHOLECYSTITIS OF RABBITS*

BY MALCOLM J. HARKINS, V.M.D., PHILADELPHIA, PA.

REPORTS of the routine bacteriologic examination of bile have included, from time to time, the isolation of the pneumococcus. These reports with the knowledge that, as a rule, bile is highly bacteriolytic for the pneumococcus have been accepted with more or less reservation; for, in the instances reported, no explanation is offered why it is possible for the pneumococcus to exist.

This institute[1] has recovered on three occasions, the pneumococcus from human bile, and like findings in a similar number of cases have been reported by Richey[2]; one isolation from a gall bladder in which the bile had been supplanted by purulent material, and in the remaining two, the pneumococcus was recovered from the gall bladder wall, the bile, and the nucleus of a calculus. Piersol and Bockus[3] refer to the isolation of the pneumococcus from bile, but in their own investigations did not find it.

Naturally in view of these findings the thought suggested itself,—is the bile from gall bladders infected with the microorganisms commonly found in cholecystitis, such as the streptococcus, Staphylococcus aureus, Staphylococcus albus and B. coli bacteriolytic for the pneumococcus? In general, the procedure adopted to meet this question was to produce a cholecystitis in rabbits by the intracystic injections of the above-mentioned microorganisms, and after an interval of three weeks, determine the pneumococcolytic activity of the bile. The microorganisms selected were all isolated in this institute from pathologic human bile except the pneumococcus, and were cultivated on 5 per cent human blood agar in tubes inoculated over the entire agar surface from a hormone broth culture, and incubated at 38° C. for twenty-four hours. The resulting growth was suspended in physiologic salt solution so that each c.c. represented two billion microorganisms. Cultures were made of these suspensions to be assured of their viability and intracystic injections of 0.2 c.c. made in the respective rabbits. The rabbits varied in weight from two to two and one-half kilos.

The direct injection of the gall bladder was made by following the technic of Beckwith[4] which is briefly as follows: After securing the animal, the site of operation of the abdomen was prepared under rigid aseptic procedure. Ether was administered and an incision approximately one inch long was made to one side of the median line with its anterior end at the base of the sternum. With retractors and sponges, the gall bladder was revealed in its cleft. With smooth blunt forceps the gall bladder was gently drawn forward and injection of the bacterial suspension made with an ordinary No. 27 gauge needle. No leakage

or bleeding occurred as the amount injected, 0.2 c.c., did not cause any appreciable intracystic pressure. The abdominal incision was then sutured and the wound treated with general surgical asepsis.

By this technic, of all rabbits injected, only one died as a direct result of the operation and this rabbit never fully recovered from the anesthetic. Three weeks later, all infected rabbits and two apparently normal rabbits were chloroformed, the bile withdrawn and placed in sterile test tubes. To each bile sample was added a two billion per c.c. suspension of pneumococci, Type I, in the proportion of 0.2 c.c. to every cubic centimeter of bile and incubated for one hour in a water-bath at 38° C. At one-half hour intervals 0.1 c.c. was removed and cultured on 5 per cent human blood agar plates. Table I gives the results of this work in detail.

TABLE I

PNEUMOCOCCOLYTIC ACTIVITY OF BILE FROM INFECTED GALL BLADDERS OF RABBITS

RABBIT NO.	CHOLECYSTITIS PRODUCED BY INTRACYSTIC INFECTIONS	PNEUMOCOCCOLYTIC ACTIVITY OF BILE	
		ONE-HALF HOUR	ONE HOUR
47	Streptococcus mitis	Absent	Absent
50	Streptococcus mitis	"	"
51	Staphylococcus aureus	"	"
52	Staphylococcus aureus	"	"
53	Staphylococcus albus	"	"
22	Staphylococcus albus	"	"
12	B. coli communior	"	Present
62	B. coli communior	"	"
37	Uninfected control	Present	"
84	Uninfected control	"	"

From Table I, it will be observed that bile from rabbit gall bladders infected experimentally with the Streptococcus mitis, Staphylococcus aureus, and Staphylococcus albus loses its pneumococcolytic action; while in bile from B. coli communior infected gall bladders, the pneumococcolytic action, while somewhat delayed, is present at the end of an hour. Compared with the action of normal rabbit bile where the pneumococcolytic action was prompt, these results are quite in contrast.

Injection of 0.2 c.c. of a two billion per cubic centimeter suspension of pneumococci into the gall bladder of a living rabbit, infected six weeks previously in the same manner with B. coli communior, confirmed these results in so far as the experiment was carried. In brief, a cholecystitis was produced by intracystic infection with B. coli communior isolated from the colon of a rabbit dying during an epidemic of infectious diarrhea; six weeks later the gall bladder was again exposed and 0.2 c.c. of a two billion per cubic centimeter suspension of pneumococci injected. One-half hour later the bile was withdrawn in a sterile capillary pipette and cultured on 5 per cent human blood agar plates and incubated at 38° C. for forty-eight hours. Many colonies of pneumococci and B. coli communior developed. The bile of two living normal rabbits, receiving intracystic injections of a similar amount of pneumococci suspension, showed no growth on culturing after the same length of time.

normal serum alone was added. Each of the lots were filled into test tubes in 10 c.c. amounts. The procedure was done in a sterile manner and on incubation for forty-eight hours at 38° C. the media remained sterile.

One tube of each lot was inoculated with the following microorganisms; Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus, and B. coli communior. These cultures were incubated for forty-eight hours at 38° C. and an abundant growth of each microorganism developed. Uninoculated tubes of each media were incubated at the same time. The cultures were then centrifugalized and the clear supernatant removed. The pneumococcolytic activity of the supernatant fluid of each culture in the two media, was then determined with the following results:

1. The pneumococcolytic activity was present in the bile medium culture fluids of the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus and B. coli communior.

2. The pneumococcolytic activity was not present in the culture fluids of the same microorganisms in the medium without bile; colonies of pneumococci being quite numerous on the plate cultures, but not as numerous as found on the plate cultures of the uninoculated medium without bile control nor in proportion to the number of pneumococci added.

3. The uninoculated bile medium was highly pneumococcolytic, the turbidity produced by the suspended pneumococci disappearing within a few minutes after adding, while the uninoculated medium without bile had apparently no pneumococcolytic activity, numerous colonies of pneumococci appearing on the plate cultures.

THE EFFECT OF THE PRESENCE OF LEUCOCYTES ON THE PNEUMOCOCCOLYTIC
ACTIVITY OF BILE

It has been suggested that possibly the leucocytes (pus cells) often found in cholecystitis were responsible for the loss of the pneumococcolytic action of the bile and that if a heavy suspension of leucocytes was added to normal rabbit bile they would affect this result. Accordingly the pneumococcolytic activity of 1 c.c. of sterile normal rabbit bile was determined as previously described and found to be present. A heavy suspension of washed rabbit leucocytes was prepared, and to 1 c.c. of rabbit bile 0.2 c.c. of the concentrated leucocyte suspension was added, the tube shaken and incubated in a water-bath at 38° C. for one hour. At the end of this time the pneumococcolytic activity of the bile was again determined and still found active.

DISCUSSION

It is appreciated that in the many reports in the literature on the bacteriology of human bile, the isolation of the pneumococcus is rare, but the fact remains that it has been isolated from a medium that has marked bacteriolytic action against it and it is to be regretted that no study was apparently made of the bile from which it was isolated, which, undoubtedly, would have given much valuable information. It is realized that the number of human bile specimens tested for pneumococcolytic activity was small; yet, this number may at least

be considered as a confirmation of the above reports and also as an index of the frequency of the existence of this condition.

It is evident from the preceding work that some change takes place within the gall bladders of rabbits infected with the Streptococcus mitis, Staphylococcus aureus, and Staphylococcus albus, which destroys the pneumococcolytic activity of the bile and delays this action in bile from gall bladders infected with B. coli communior for a period of three weeks. *In vivo* this work was confirmed in one rabbit infected with B. coli communior controlled by two normal rabbits.

The literature on the germicidal properties of hepatic duct and cystic bile for microorganisms other than the pneumococcus and streptococcus, is quite voluminous and in an excellent review given by Neilson and Meyer[5] one is quite impressed by the differences of opinion, four different properties being ascribed to bile *in vitro*, namely; it favors the growth of common bacteria, except pneumococci and streptococci; it is indifferent or it may be inhibitive, or it may even be germicidal. While no definite explanation was noted in the literature explaining the germicidal properties of normal bile for the pneumococcus, Avery and Cullen[6] attribute the solubility of the pneumococcus to enzymes contained within the organism, which, it is interesting to note, acts best at a P_H concentration most favorable to pneumococcus growth 7.0-7.8. While these factors apply to the bile of apparently normal and immunized animals, the extent of their application to bile from infected gall bladders of rabbits is limited; because from the findings here reported and other reports of bacteriologic examinations where pneumococci were noted, the germicidal and bacteriolytic effect on pneumococci was not present.

That the suspension of the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus, and B. coli communior in normal rabbit bile; the growth of the same microorganisms in a medium rich in bile; likewise, the suspension of leucocytes (pus cells) in normal bile did not remove the pneumococcolytic activity is suggestive that the ideal conditions exist only within the gall bladder or its walls, the infective microorganism producing there a pathologic condition interfering with the normal functions of the gall bladder; such as the concentration of the bile; conversion of the bile salts to other substances, and thus removing from the bile its germicidal and bacteriolytic power to act on pneumococci.

CONCLUSIONS

1. A bacterial cholecystitis was produced in rabbits by the direct injection of the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus, and B. coli communior into the gall bladder.

2. Bile from experimental bacterial cholecystitis of rabbits, produced by the above microorganisms except B. coli communior, loses its pneumococcolytic activity.

3. The pneumococcolytic activity of bile from cholecystitis of rabbits produced by B. coli communior, while present is somewhat delayed, and *in vivo* this result was confirmed.

4. Of twenty-four human bile specimens tested for pneumococcolytic activity, all were found to possess this characteristic.

5. Suspension of the Streptococcus mitis, Staphylococcus aureus and albus, B. coli communior, and leucocytes in normal rabbit bile did not remove the pneumococcolytic activity. Also cultures of these microorganisms in 10 per cent bile medium failed to remove this characteristic.

REFERENCES

[1]Personal communication from Dr. J. A. Kolmer, Head of the Department of Bacteriology and Pathology, Research Institute of Cutaneous Medicine, Philadelphia, Pa.
[2]Richey, de W. G.: The Bacteriology of Human Cystic Bile, Pennsylvania Med. Jour., 1922, xxvi, 4.
[3]Piersol, G. M., and Bockus, H. L.: A Study of the Bile Obtained by Nonsurgical Biliary Drainage with Especial Reference to its Bacteriology, Am. Jour. Med. Sc., 1923, clxv, 486.
[4]Beckwith, T. D.: Direct Injection of B. Typhosus into the Gall Bladder, Jour. Infect. Dis., 1922, xxxi, 468.
[5]Neilson, N. M., and Meyer, K. F.: The Bacteriostatic and Germicidal Properties of Bile, Jour. Infect. Dis., 1921, xxviii, 542.
[6]Avery, O. T., and Cullen, G. E.: Studies on the Enzymes of Pneumococcus, I Proteolytic Enzymes, Jour. Exper. Med., 1920, xxxii, 547.

A SIMPLE AND RAPID QUANTITATIVE TEST FOR ALBUMIN IN URINE*†

By WILLIAM G. EXTON, M.D., NEW YORK CITY

INTRODUCTION

SINCE 1770, when its presence in urine was first demonstrated by Cotugno, the test for albumin has probably been performed oftener than any other chemical or clinical test. The deficiencies in our present knowledge of albumin excretion must, therefore, be laid to the lack of a satisfactory clinical quantitative method, because the statistical and clinical advantages of placing albumin determinations on a quantitative basis are, of course, self-evident. Particularly striking, at the present time, is the great contrast between the practical absence of data on albumin excretion and the vast accumulation of intensively worked out data on sugar excretion, which is daily increasing our knowledge of carbohydrate metabolism. These considerations, together with the experiences gained by typing albumin excretion by means of methods, shortly to be published,[1] led to the conclusion that a simple, accurate, and rapid method of measuring albumin in urine was something in the nature of a necessity.

Experimental work directed towards finding such a method was instituted by reinvestigation of the qualitative albumin tests in common use, because a test was aimed at from the outset which would require no more time or trouble for its performance than Heller's or the heat test which physicians for so long a time have been accustomed to employ. The possibilities of the

nitric acid ring and of the heat test for quantitative work were exhausted, however, without success, notwithstanding the many modifications which have been proposed for the purpose. The reasons why such failure is inevitable are easily understood when a method of attack such as ours is employed. This differed from those of other investigators in that from the beginning all experiments were made in duplicate with solutions of known protein concentration in water and urine. As regards Heller's nitric acid ring test, it may be noted in passing that no stratification, or layer, test can be made to yield reliable quantitative results with any technic whatsoever. Furthermore, the compositions of different specimens of urine influence the velocity of development and intensity of the rings which may form and so lead to false deductions. Thus it was observed that serum protein in distilled water solutions exhibited rings in concentrations of one part in 30,000 parts of water, but that the same concentrations in different samples of albumin-free urine often failed to show a ring in concentrations as low as one part in 12,000 parts of urine. With the heat test, it was found that a number of uncontrollable factors, physical and chemical, such as hydrion concentration, kind and amounts of salts in solution, intensity of heat applied, etc., affect the character of the coagula and prevent the achievement of uniform results. It is also worthy of mention that there are other variables, such as the presence of certain drugs and preservatives, which detract from the reliability of both Heller's and the heat tests.

Gravity tests, like Esbach's, which take hours to complete, or tests depending upon centrifugalization, were not considered within the scope of our work on account of previous findings of inaccuracy and because a test was wanted which would not only be free of the personal equation of the analyst, but which would also give prompt results with an irreducible minimum of manipulation and apparatus.

The precipitation characteristics of different protein precipitants were next studied, with the intention of selecting the one which promised to best fulfill the conditions necessary for measuring protein in urine as understood by us. In the order of their importance these are:

1. Specificity of precipitation for the proteins of present clinical interest in urine.
2. Quantitative precipitation.
3. Constancy of reproducibility.
4. Promptness of precipitation.
5. Sensitivity.
6. Suitability of precipitate for optical measurement.
7. Stability of reagent.
8. Simplicity of technic.
9. Elasticity of time element.
10. Economy.

The result of the trials favored sulphosalicylic, or salicyl-sulphonic acid, as it is sometimes called in the literature.

Older experimenters called attention to the fact that when sulphosalicylic acid is added to some urines an opalescence or cloudiness develops, which disappears upon warming, and they attributed such instances to the presence

of albumoses or peptones. In our own experience of 200,000 specimens tested with sulphosalicylic acid all such cases were found to be caused either by conjugates, by resinous substances, by protamines, or spermines, or similar large molecules associated with the presence of semen in urine. Cloudiness caused by substances other than albumin may be quite pronounced and easily mistaken for albuminous clouds, therefore, sulphosalicylic acid is specific for the proteins of clinical interest in urine only when used under certain definite conditions, to which I have called attention in previous papers.[2]

Every method of applying sulphosalicylic acid to urine should provide for warming tests which become cloudy. A little heat causes the cloudiness to disappear as the nonalbuminous substances go back into solution when warm. Albuminous clouds, on the contrary, when once established, remain unchanged, whether the test be cold, warm or boiled. They are easily distinguishable from the precipitate formed by Bence-Jones' protein with sulphosalicylic acid, because Bence-Jones' precipitates disappear upon boiling and are always very dense and appear exactly like curdled milk. The presence of Bence-Jones' protein should always be suspected whenever an albumin test yields an unusually dense precipitate of curdled appearance. When used under such conditions, sulphosalicylic acid may safely be regarded as being more specific for the proteins of clinical interest in urine than either Heller's or the heat test.

PRECIPITATION

The test to be described is grounded upon the albumin test previously described by me, which has been in routine use in the Prudential and other laboratories for years. Experience has confirmed its accuracy and reliability as a qualitative test, and satisfactory quantitative results have been uniformly obtainable with it in conjunction with the scopometer.[3] The test is empyrical but takes cognizance of the variables which arise in the treatment of urinary proteins with sulphosalicylic acid because the resulting precipitates are influenced by a number of factors. These factors include:

1. The concentration of sulphosalicylic acid.
2. The concentration of protein.
3. The relative dilutions of protein and acid.
4. The natures and proportions of different proteins (i.e., globulin-albumin).
5. Unknown substances in urine which may be adsorbed.
6. The kinds of salts present.
7. The quantities of salts present.
8. Temperature.
9. Time.

As regards concentration and dilution of precipitant and protein, it was assumed *a priori* that the employment of equal volumes of precipitant and specimen would make for the utmost technical simplicity and also greatly simplify any calculations which become necessary. It was therefore experimentally determined that the optimum concentration of sulphosalicylic acid for precipitating urinary proteins in this way was between 4 per cent and

7 per cent, and 5 per cent was adopted for the method as being ample for the precipitation of any possible concentration of albumin which might be encountered in urines and as being sufficiently acid to meet all likely reaction variables. Numerous specimens of different types of urine were then taken at random and tested with sulphosalicylic acid in three different ways: (A) equal parts of urine and 5 per cent sulphosalicylic acid; (B) one part of urine and twenty-four parts of 2 per cent sulphosalicylic acid; and (C) one part of urine and three parts of 3 per cent sulphosalicylic acid. Comparisons which were run on the same urine specimens with tests A and B showed that, when measured by comparable standards, 30 per cent to 40 per cent of the various specimens exhibited very considerable discrepancies in the protein values obtained with A and B tests. It was also the rule in every case in which a discrepancy was found that the B test gave higher results than the A test. When the protein was isolated from such urines, and estimated gravimetrically, or by nitrogen determinations, the checks, with a single exception, were closer to the A test, made with equal parts of urine and reagent. It was also found in every instance in which there was such a discrepancy that further dilution would magnify it. Runs were also made on the same specimens, using equal parts of 5 per cent sulphosalicylic acid and urine (A), and one part of urine to three parts of 3 per cent sulphosalicylic acid (C). As was to be expected, the results showed the discrepancies to be fewer and less than when one part of urine was treated with twenty-four parts of 2 per cent sulphosalicylic acid (B).

Here, again, however, when the discrepancies were checked by nitrogen

TABLE I

ONE SERIES OF DISCREPANCIES FOUND BY TREATING URINES WITH SULPHOSALICYLIC ACID IN THREE DIFFERENT WAYS

DISCREPANCY NUMBERS	MILLIGRAMS OF ALBUMIN PER 100 C.C. URINE			GRAVIMETRIC AND KJEL- DAHL'S CHECKS	NOTES
	A	B	C		
1	75	200	—	72	A—2 c.c. urine: 2 c.c. sulphosalicylic acid 5 per cent
2	30	75	—	25	
3	50	100	—	55	B—1 c.c. urine: 24 c.c. sulphosalicylic acid 2 per cent
4	25	75	—	21	
5	50	150	—	55	C—2.5 c.c. urine: 7.5 c.c. sulphosalicylic acid 3 per cent
6	20	200	—	31	
7	40	200	—	50	Specimens, nos. 1 to 10 and 16 and 17, were
8	160	300	—	170	checked by the gravimetric method. The
9	40	100	—	50	urines were adjusted to P_H 4.6–4.8,
10	50	100	—	64	heated in boiling water, and centrifuged.
11	50	100	—	62	The coagula were then washed and
12	80	100	—	92	weighed
13	120	200	—	120	Nos. 11–15 were checked by Kjeldahl's
14	300	1000	—	336	method. The albumin was isolated in the
15	125	200	—	113	same way as in the gravimetric checks,
16	135	150	150	105	and nitrogen determinations made (N× 6.4).
17	60	125	100	77	Specimen No. 15 when diluted 1:74, and B test again applied, read 1000 mg. per 100 c.c.

determinations the results were always closer to the values obtained by testing with equal parts of precipitant and urine.

These results could not be taken as meaning otherwise than that the relative dilution and concentration of urine and precipitant greatly influence the optical effects by which estimations are made, and that the employment of equal volumes of urine and precipitant not only offered by far the simplest technic but also led to greater quantitative accuracy than any other method of precipitating protein in urine with sulphosalicylic acid.

If the same solution of sulphosalicylic acid be added to similar concentrations of albuminous urine and aqueous solutions of serum protein, it will be observed that the precipitates show differences. The urinary precipitates appear to be finer and in better equilibrium, as evidenced by the fact that they stay appreciably longer in suspension before flocculating. This offers at least a partial explanation of the failure of nephelometry and turbidimetry to yield consistent checks with freshly prepared protein standards. In urine the difference suggests that the effects may be explained by its higher viscosity with greater internal friction and the presence of something like protective colloids. Probably both of these combine with salt effects. In order to eliminate, if possible, the variables encountered in urine so as to get as uniform precipitation as possible, attempts were made to modify the character of the sulphosalicylic-protein precipitate. These demonstrated that the presence of sodium sulphate modifies the character of the precipitate obtained with albuminous urine and aqueous solutions of protein in such a way as to make them more alike. Sodium sulphate was therefore added to sulphosalicylic acid in reagent form.

<center>ESTIMATION</center>

The next step was directed towards finding the easiest possible method of measuring the precipitates or turbid suspensions developed with the reagent and albuminous urine. No standard of measurement which did not promise to be stable or permanent was considered. Originally it was thought that some kind of cloudy glass in the form of tubes or a wedge could be found or made to answer the purpose in conjunction with some sort of contrivance for gauging it against the cloudiness of the urine tests. When it became necessary, however, to confess the futility of thorough search and efforts along these lines, a different angle of approach was sought and numerous attempts were then made to find or concoct some sort of emulsion, or sol, or inorganic precipitate—in fact, anything which might work as a satisfactory standard for measuring turbidities.

When this line of attack presented no solution the possibilities of gels like silicic acid and gelatine were next exploited, and Mr. Clyde Brockett, of the research laboratory of the Eastman Kodak Co., succeeded in making a series of standard tubes which matched beautifully with the urine tests. These were shown at the San Francisco meeting of the Society and proved satisfactory in every other way but permanency. The gels liquefied sooner or later and settling of the particles spoiled the tubes.

With experience it became plain, practically as well as theoretically, that any tubes intended for comparison standards must be made under exactly

the same conditions as the protein in urine is precipitated, or under the closest approximate conditions which could possibly be contrived. A return was therefore made to our earliest experiments in preparing the fresh standards for nephelometry, because a possibility of finding some way of manipulating these into a permanent condition was still open. Clear, albumin-free urine was boiled and series of standard tubes were made by adding to each a known concentration of protein and precipitating in precisely the same way as in making the urine tests, and sealing the tubes immediately. These standards also proved satisfactory in every way except permanency. They were easy to reproduce and some sets made in this way remained serviceable for months. Sooner or later, however, changes took place in the urine which spoiled the tubes.

Trials were then made in the same way with aqueous solutions of different proteins, and what was considered a partial success was thus achieved. Some sets of these tubes kept without apparent change for six months or more and were used under careful control in the Prudential laboratory and exhibited at meetings as long ago as 1918. The changes in the aqueous tubes were of a different order from those which took place in the urine standards. The change in the urine tubes was striking. They would suddenly darken and become very turbid—generally overnight. In the aqueous tubes, on the other hand, deterioration took place slowly and gradually, and the only kind of changes which it was possible to detect in them affected the condition of the precipitates. The particles would either lump, so as to take on a granular appearance, or by a distinctly different form of coalescence the particles would agglomerate in shreds. It was often possible to break up the agglomerates by violent agitation and thus prolong the usefulness of the tubes.

It was found practicable to reproduce such tubes easily and surely, and as no changes were perceptible other than the coalescence or agglomeration of the particles, our problem finally seemed to narrow itself down to contriving some means of preventing the eventual coalescence or agglomeration of the particles. The effects of substances which raise viscosity and internal friction, and of protective colloids, were therefore studied because they seem to be present in urine. At the same time experiments were also made with a number of dyes which were known to be adsorbed by protein. Of these, the indicator dye, bromphenol blue, was tried, at the suggestion of Dr. H. Sharlit, of the Harriman Research Laboratory, because it has a yellow color in acid solutions which is not unlike that of normal urine. Bromphenol blue apparently stabilizes the sulphosalicylic-protein precipitate by being adsorbed on the particles, which take on its yellow color. This effect increases the visibility of the particles or cloudiness and makes the test more sensitive. The dye also acts to diminish the color contrasts between urine and standard tubes, and gives the additional advantage of eliminating opalescence, which is sometimes bothersome in comparing undyed turbidities.

Starch and gum arabic apparently are not affected by sulphosalicylic acid, and when solutions of serum protein are made with either of these, the resulting precipitate has every appearance of being like that of albuminous urine of the same concentration which has been treated with the reagent con-

taining the dye. By including gum arabic in the serum solutions, and by precipitating with a reagent containing bromphenol blue, sets of standard tubes have been made which have kept for well over a year without perceptible change in color or difference in cloudiness values. These have been considered to meet the permanency requirement.

ALBUMINIMETRY

It has been customary to compare samples of cloudy liquids by either holding them up to the light or by viewing them against a black background. Individual preferences usually decide the matter, although it is generally accepted that the denser dispersoids are best compared by transmitted light, and the finer turbidities against a black background. In watching the work of the technicians in the Prudential laboratory, it was noted that difficulties arose whenever it was sought to have all of them make turbidity comparisons in the same way. Each one seemed to favor some particular spot in the laboratory where he thought that the lighting was best adapted for distinguishing differences in opacity. Some technicians preferred to compare three tubes instead of two by placing the unknown sample between the two standards nearest to it, viz., the next denser and the next lighter. Undoubtedly such practice tends to hasten decision and increase the delicacy and accuracy of reading. Some workers, as is well known, experience more difficulty than others in making turbidity comparisons and manifest indecision by trying out more standards and by trying them oftener and under more diverse lighting conditions and against various backgrounds. In the effort to improve and make more uniform matters of technic, it was learned that inaccuracy and indecision were usually traceable to one or more of three different causes:

1. Inequality of lighting the tubes.
2. Reflections between the tubes.
3. Differences in the colors of standard tubes and urine tests due to the variable colors of urine.

There are other factors which probably play a part in causing hesitation at times, but experience has shown that when those which have been mentioned are eliminated, even novices are able to make correct readings quickly and without indecision.

As some sort of an arrangement was necessary for conveniently keeping and handling the standard tubes, it was decided to design a rack and incorporate in it a simple device for albuminimetry which would prevent reflection between the tubes and provide for equality of lighting them. (See Fig. 1.) In addition to these, an opacitimetric gauge was proved experimentally and found to facilitate and standardize readings and has been provided by interposing a broad, black line on a white background behind the tubes. When light falls on the tubes and passes through the turbid media in them, the visibility and shading of the black line show changes which are directly proportional to the opacity of the media through which light reaches the black opacitimetric line. Very definite turbidity comparisons are thus made practicable, and the method is sensitive to a degree which allows the use of standard tubes approximating each other so closely that similar readings would be otherwise

impossible without the aid of a complicated and expensive optical instrument. This clinical albuminimeter permits one to hold the tubes up to the light (nephelometry), if one prefers to match that way; or, by an instantaneous shift, to mark the shading of the black line back of the tubes by having the light fall over the shoulder of the observer; or, better yet, by having the light from overhead fall on the tubes, held at an angle in front of a light background, such as a towel or piece of paper laid on the table (opacimetry). Worked in the lower ranges, this device will register differences as minute as those represented by one part albumin in 50,000 parts of urine without difficulty in either artificial or sunlight.

It goes without saying that the suspensions in the standard tubes should always be made homogeneous before matching, by turning the tubes. If a practice be made of keeping each tube in its proper order, or pocket, in the
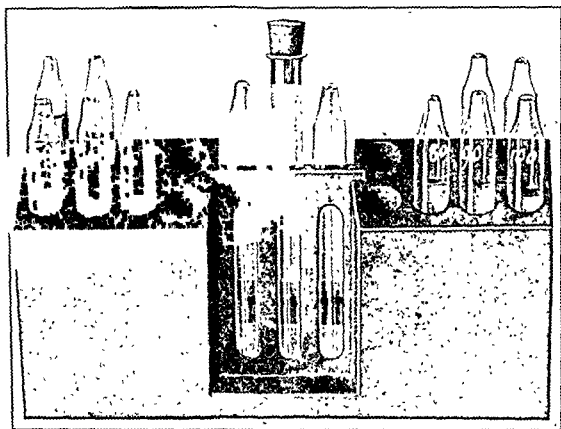
Fig. 1.—Exton's clinical albuminimeter In order to show method of reading opacities the light filter has been removed.

rack when handling them, a little experience will lead to automatically picking the right tube and replacing it in the right pocket. For convenience when making several tests, empty pockets are provided for a few extra tubes.

Examination with the spectroscope shows that urochrome, or the coloring matter of normal urine, absorbs blue light; darker urine also absorbs part of the green light of the spectrum, and the absorption of the bromphenol blue in acids as used in the reagent is similar, therefore, if the urine tests and standard tubes are viewed through a light filter which cuts out the light that would be partially absorbed by urine and which lets through only the light which is unaffected by the color of the urine, differences in color between standard tubes and urine tests disappear. Yellow and red light filters of selected spectral transmission are available which will satisfy these requirements, and are conveniently and with advantage combined with the other fea-

tures of the clinical albuminimeter. This simple expedient is efficient in eliminating practically all of the difficulties encountered in comparing turbidities which arise from differences in color, and a trial of the experiment will demonstrate that the differences in color between water and urine and bromphenol blue reagent are practically annihilated when samples of these are viewed, side by side, at the same time, through a filter of proper spectral transmission. The combination of comparing three. tubes with equal lighting and without reflections, with opacitimetric black line and light filter, avoids every difficulty which has arisen within our experience in measuring cloudiness, and, as embodied in the clinical albuminimeter,* affords an unexpectedly easy, certain, precise, and sensitive means of comparing and measuring opacities.

TIME ELEMENT

The time after precipitation when the opacities or suspensions are read is a very important factor in the accuracy of all albumin tests. In turbidimetry, with freshly prepared standards, it has been held to be the best practice to make readings at the moment before the precipitates begin to flocculate in order to get as complete precipitation as possible and yet avoid the interferences due to coalescence and settling of the particles. In the case of sulphosalicylic-protein precipitates, it is known that the velocity of precipitation depends upon the concentration of protein in the specimen. The correct timing of an unknown concentration of protein is, therefore, a practical impossibility. To try to meet this difficulty by fixing the same arbitrary time for reading all concentrations is a makeshift which becomes very troublesome because it obligates the analyst to mark time by the clock in order to make his comparisons or readings at a certain definite instant, before or after which readings will give inconsistent results. This method of timing also has the objection that it precludes warming the tests, which sacrifices the specificity for albumin,—perhaps the greatest advantage of sulphosalicylic acid.

It was possible to study some phases of precipitation and behavior of particles in suspension with the scopometer, and by this means observations were made on the precipitation of protein with our reagent, with reference to time and reducibility. As to time, it was learned that precipitation goes on faster as protein concentration increases. As to reproducibility, it was found that if precipitation be allowed to go on beyond the flocculation point, homogeneous suspensions could again be secured in all concentrations by simply turning the tubes upside down, and repeated scopometric measurements of the turbidities produced by different concentrations of protein established the reproducibility of such suspensions even after the precipitates had been settled in the bottoms of the test tubes for hours.

These results pointed to the practicability of applying heat in order to accelerate precipitation and make the test specific for albumin. They also showed a way to. rid the test of the troublesome time element with its burden of clock watching. By having the values of the standard tubes represent complete precipitation of the protein in them, it is only necessary to let the urine

*Standard tubes, reagent, and rack may now be obtained from Lehn and Fink Inc., of New York City.

tests stand until precipitation is practically complete. They can then be read against the standards at any convenient time thereafter.

ACCURACY

Under the usual conditions of clinical work the experimental error of the method should be within five mg., or one part albumin in 20,000 parts of water. This degree of accuracy is attainable by waiting five minutes, or longer, before warming and reading the test. If haste be not necessary it is handier to let the tests stand until some later time when it is convenient to read the results. If greater accuracy is desired, dilute the specimen so that the concentration of protein falls within the range of 20 mg. to 50 mg. per 100 c.c., and let precipitation go on in an incubator for several hours. Then read the homogeneous suspension against intermediate standard tubes representing 5 mg. difference in value. By careful work in this way results are possible within an experimental error of 2 mg., or one part albumin in 50,000 parts water.

REAGENT

The precipitant is used in the form of a single solution, which appears to keep indefinitely. It contains sulphosalicylic acid (Eastman), sodium sulphate (crystals), and bromphenol blue (clear, saturated, 0.4 per cent watery solution).

To prepare: Dissolve 50 grams of sulphosalicylic acid* and 10 grams of sodium sulphate in about 800 c.c. of distilled water, and add 25 c.c. of the dye. Make up to one liter and filter through acid-washed paper (Whatman No. 40).

THE STANDARD TUBES

Convenience is gained by limiting the number of standard tubes to an adequate minimum, and every clinical requirement is met by having them differ from one another by 10 mg. of albumin, or one part in 1,000. For closer work it is just as easy to make intermediate tubes and read them in the clinical albuminimeter. The sets of standards used in the Prudential laboratory for routine work range from a blank to 100 mg. of albumin, so that they comprise a series of twelve tubes, representing 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg. protein per 100 c.c. We have adopted the mille per cent expression for values because in practice it has proved simpler, safer, fitter, and more convenient than any other. Urine which reads more than 100 mg. is diluted with water, either before or after precipitation. Urine which reads between the blank and 5 mg. or 10 mg. tubes, can be read satisfactorily, because error in the low ranges is greatly diminished and clinically negligible. Some may find it easier, however, to increase the proportion of urine to reagent, and read against standards of higher values.

Preparation of the tubes is simple but demands careful work for precise reproduction.

It is very essential to work with scrupulously clean apparatus and to take every possible precaution to exclude foreign matter like dust, etc.

TABLE II

REPRODUCIBILITY OF STANDARD TUBES MADE FROM BLOOD OF DIFFERENT ANIMALS AND AT DIFFERENT TIMES

I	F	E	D	M	N	O	P	NN	R	S
100	100	100	90	100	100	100	100	100	100	100
80	80	80	70	80	80	80	80	80	80	80
60	60	—	—	60	60	60	60	60	60	60
50	50	50	45	50	50	50	50	50	50	50
40	40	40	—	40	40	40	40	40	40	40
30	30	—	35	30	30	30	30	30	30	30
25	25	25	25	25	25	25	25	25	25	25
20	20	20	20	20	20	20	20	20	20	20
15	15	—	—	15	15	15	15	15	15	15
10	10	12	9	10	10	10	10	10	10	10
5	5	5	—	—	5	—	5	—	5	5

I—Sheep serum.
F—Sheep serum.
E—Sheep serum diluted with normal urine.
D—Pig serum.
M—Steer plasma.
N—Steer plasma diluted with 1 per cent NaCl to the 100 mg. protein concentration.
O—Steer serum.
P—Sheep serum.
NN—Sheep serum diluted with gum arabic solution.
R—Sheep plasma.
S—Sheep serum from same animal as R.

The serum, preferably sheep, must be as fresh and free from hemoglobin as possible, because we have a very distinct impression that old serum, even when kept in a chilled condition, does not make as satisfactory tubes as does fresh serum. We have found it best to collect the blood in clean, shallow pans, and let it clot well before decanting the serum into wide-mouth containers. The clear serum is promptly and thoroughly centrifuged (more than once at high speed), and samples are taken for protein estimations by Kjeldahl nitrogen methods (Nx6.4), or more conveniently by diluting and precipitating with the reagent and reading the scopometer scale. The serum is then diluted with 5 volumes of 2 per cent NaCl solution. Further dilutions are made with distilled water on the basis of the known protein content so as to get a stock solution containing 200 mg. protein per 100 c.c. One per cent and 0.5 per cent solutions of purest, finely ground gum arabic (i.e., clear) should be ready at hand. The desired series of protein concentrations is now obtained by diluting portions of the 200 mg. stock with distilled water and the gum arabic solutions. The proportions of gum arabic and water should be such as to make a 0.5 per cent solution of gum arabic in each protein tube.

We use 125 mm. by 12.5 mm. clear glass stock test tubes. They are calibrated by selecting those in which equal volumes of water stand at the same level, and cleaned with bichromate-sulphuric acid mixture. They are then washed several times in distilled water and dried in the oven. About fifteen tubes are needed for a set and these are constricted about one inch from the mouth by drawing them out in the flame. This helps to expedite neat sealing. Small funnels (Fig. 2) are also prepared from the test tubes by drawing them out in the flame to a diameter that will go through the constrictions of the tubes. These facilitate filling and prevent wetting the constrictions.

Four c.c. of the desired protein concentrations are then poured into the tubes through the funnel, and filling is completed by adding 4 c.c. of the reagent. The tubes are sealed immediately. This is easily done by tapping

off the tops of the tubes at a file scratch on the constrictions and slowly revolving the open points in the flame of a blast lamp.

When cool, gently invert the tubes, to insure complete mixing, and be sure to turn the tubes gently several times a day during the first ten days. Diamond ink answers for marking the tubes. It seems scarcely necessary to caution against letting the tubes lie lengthwise, or stand upside down, and, of course, exposure to extremes of temperatures should also be avoided.

An occasional set of tubes will turn out less perfectly than others, due to unexplained differences in sera. Such anomalies become apparent in forty-eight hours. Sets of tubes made at bimonthly intervals over a period of eighteen months have been carefully and frequently compared with reference to optical values and the condition of the suspensions, and have been found to check perfectly and keep without apparent change.

The matter of color permanency was watched with special interest because it was feared that the dye would fade. No changes, however, have been
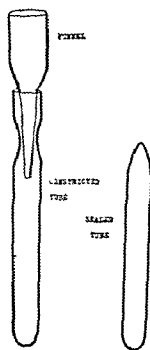
Fig. 2.—Glass parts used in making the sealed standard tubes.

detected. In this connection it may be of interest to note that we subsequently and accidentally learned that dyers make empirical use of the fact that dyes which show absorption in the ultraviolet region of the spectrum are made fast by mixing with them some salt having a similar absorption. Bromphenol blue and sodium sulphate show absorption in the ultraviolet.

In the course of the work it was necessary to make hundreds of experimental tubes, and I am happy to record my appreciation of the careful assistance given throughout by John Huizer, who is also entitled to credit for the technic of filling and sealing the tubes. I desire also to gratefully acknowledge the very material help in many ways of Anton R. Rose, who, with the assistance of Fred Schattner, thoroughly checked our earlier experiments.

DIRECTIONS

The test is calculated so that the cloudiness or opacity which results from mixing equal parts of urine and reagent denotes the number of milligrams of

albumin in 100 c.c. of urine. Clean test and standard tubes are essential for making the test, and cloudy urine should be cleared, if possible, by filtering, or by centrifuging and testing the supernatant urine. *Add 2 to 3 c.c. of reagent to a like amount of urine and let stand five minutes, or until it is convenient to read the result with the clinical albuminimeter.* Turn the tubes gently to make the suspensions homogeneous, and warm the test immediately before reading by passing the tube in and out of the flame several times in order to avoid boiling which is undesirable except when differentiating Bence-Jones' protein. The absence of cloudiness denotes the absence of albumin in the urine, while the presence of cloudiness means that the urine contains albumin, which is then measured with the albuminimeter. *Turn the albuminimeter rack (cover on) to have the suspensions in the standard tubes homogeneous.* With the test in the middle one of the three pockets, try out the standard tubes by placing them in the pockets on both sides of the test. Comparisons of opacity or cloudiness will show which two standard tubes closest approximate the test, i.e., a denser and a lighter one. *The result is then read by a match or by taking the reading between the two tubes next higher and next lower than the test.* If the test be cloudier than the 100 mg. standard tube, dilute five or ten times with water and multiply the reading by the number of dilutions; for example, if the original test consists of 4 c.c. (i.e., 2 c.c. urine and 2 c.c. reagent), dilute with water to 20 c.c. and multiply the result by 5. Some may find it more convenient to make a fresh test and dilute the urine with water before precipitating. *To make the test on cloudy urine* which cannot be cleared, dilute a portion of the urine with an equal volume of water and read against the standard tubes; precipitate a similar portion of the urine in the regular way and read against the standard tubes; the difference between the two readings measures the protein content. Urine containing large amounts of carbonates or other alkaline salts is apt to froth when any albumin test is applied, and it is better to acidify such urine with acetic or some other acid before testing. Decomposed or extremely alkaline urine will turn blue or purple when the reagent is added to it because bromphenol blue is also an indicator on the alkaline side. Such urine should also be acidified before making the test. If the color of a specimen should happen to be very dark or unusual, dilution and the light filter will bring it within the color range of the standard tubes.*

CONCLUSIONS

A simple and rapid quantitative test for albumin in urine has been described. Every contingency which is known to arise in the examination of urine for albumin is met by the method, which requires no more time or manipulation for its performance than do the qualitative clinical tests in common use.

The specific and quantitative features of the new test impart an accuracy and uniformity to albumin determinations which make them clinically valuable to a degree hitherto impossible.

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*If a correct light filter is not available make a color control solution containing the same amounts of dye and sodium sulphate as the reagent and 5 c.c. of concentrated sulphuric acid to the liter. The control solution has the same color as the reagent and avoids the need of a light filter if substituted for distilled water when diluting the urine.

REFERENCES

1Exton, W. G.: Typing of Albumin Excretions; Meeting Am. Med. Assn., May 29, 1925.

2Exton, W. G.: A Simple and Rapid Test for Albumin; Proceedings of Association of Life Insurance Medical Directors, 1921, p. 188.

Exton, W. G.: A Simple and Rapid Test for Albumin and Other Urinary Proteins; Jour. Am. Med. Assn., Feb. 24, 1923, lxxx, 529-530.

3Exton, W. G.: A Simple and Rapid Method for the Determination of Albumin in Urine with Preliminary Presentation of the Scopometer; Proceedings of the Second Annual Convention of the American Society of Clinical Pathologists, June 25-26, 1923, JOUR. LAB. AND CLIN. MED., March, 1924.

Exton, W. G.: The Scopometer: An Instrument for Measuring Color, Cloudiness, and other Optical Phenomena of Liquids; Proc. Soc. Exper. Biol. and Med., 1924, xxi, 181-182.

Exton, W. G.: A New and Direct Method of Measuring the Cloudiness of Liquids (Scopometry); Transactions, Joint Meeting of the American Physical Society and the Optical Society of America, New York, February, 1925.

ON THE INFLUENCE OF ACIDS UPON THE VIABILITY OF BACTERIA*

By HIDETAKE YAOI, TOKIO, JAPAN

INTRODUCTION

IT has already been clearly settled by numerous researches of preceding authors that the reaction of media has great influence upon the viability of bacteria.

The effect of reaction of media upon B. coli communis, which I employed in my experiment, has been repeatedly studied by many workers; their most important results are given in the table on page 736.

The results may be summarized thus: The width of the reaction allowing viability of B. coli lies between P_H 4.6-9.0, the optimum being in a rather weak acidity of the media.

The minute differences among the results of these authors may be probably ascribed mostly to the individuality of the strains employed in their experiments, and furthermore the methods of cultivation and composition of media as well as the temperature of incubating may have played some rôle on the different attitude of the organisms.

The methods, upon which these authors determined the death-point of bacteria, may be divided into two groups:

1. Determination of the death-point by estimating the acidity which the bacteria produced after development on media with sugars.

2. Determination of the death-point by observing the occurrence of growth in media, to which acids or buffer solution have been added. In the latter case the acidity or alkalinity of the media has been conveniently defined by the addition of HCl, or NaOH as the case may be, to the media.

		AUTHORS	P_H (DEATH-POINT)	P_H (OPTIMUM)	MEDIA	BIBLIOGRAPHY
1	1912	Michaelis and Marcora	5.0		Lactose-Broth	Z. Imm. u. ex. Therap. I Teil, (orig.) xiv, 170.
2	1917	Shohl & Janney	4.6-9.6	6.0-7.0	Urine	J. Urology, i, p. 211.
3	1917	Clark & Lubs	4.5-9.0	6.0-7.0	Broth	Jour. Bact., ii, 1.
4	1918	Wyeth	4.3		Broth	Biochem. Z., xii, p. 382.
5	1919	Cohen & Clark	4.5-9.0	6.0-7.0	Broth	Jour. Bact., iv, p. 409.
6	1920	Chambers	4.9		Glucose-Broth	Ann. Missouri Botan. Garden, vii, p. 249.
7	1921	Dernby	4.4-7.8	6.0-7.0	Autolysated broth	Ann. Inst. Past. T., xxxv, p. 277.
8	1921	Scheer	4.7		Broth	Z. Imm., xxxiii, p. 36.
9	1922	Adam		6.4-7.1	Broth with and without sugar	Centralbl. Bakt., i, Abt. Bd., lxxxvii, p. 450.
10	1923	Dernby & Näslund	4.5-9.0	6.0-7.0	Broth	Z. Imm., I Teil, xxxv, Heft 5-6, p. 450.
11	1923	Kojima	4.8		Broth with sugar	Japan J. Exp. Med., vii, p. 1029.

It is to be regretted that few have paid much attention to the kinds of alkali and acid to give the media the desired reaction, i.e., hydrogen-ion concentration. Nor can I judge from my own experiment whether it is right or not to determine the acidity only by the actual acidity. A most complicated relationship of the acids and the growth of bacteria should be expected owing to the difference of acids, i.e. anion, dissociation-constant and valency etc., which must give widely diverging effects to the bacteria according to the kinds of chemicals employed.

Kojima[1] reported that the end-reaction, namely the death-point of bacteria of media, varies very much, according to the kinds of sugar added to the culture fluid. This resulted presumably from the different products in these media. Also the difference, qualitative as well as quantitative, of the organic acids contributed to the variation of the results.[2, 3]

On the other hand Krönig and Paul,[4] 1897, gave some notes on the bactericidal action of acids in their report, and described that bacteria are affected not only by the cation, i.e., H, but also by the anion and undissociated molecules. Other authors, however, differed in their method of examination and materials in their experiments, or employed entirely different acids; accordingly their results very often show no agreement.

For instance, Bial[5] found in 1902 that the glucose fermenting power of yeast is affected by organic as well as inorganic acids, and especially that the greater the dissociation the more the fermentation is interfered with. In such a minute concentration of the acid, as he describes, the action of the anion is scarcely noticeable and so the inhibiting action must be considered totally due to the dissociated H.

In 1906, Winslow and Lochridge[6] reported that "The mineral acids, HCl and H_2SO_4, are fatal in concentrations at which they are highly dissociated. Their action runs parallel, not to their normal strength, but to the number of free hydrogen ions per unit volume.

"The organic acids, acetic and benzoic, are fatal to the typhoid and colon bacilli at a strength at which they are only slightly dissociated. The effect here appears to be due to the whole molecule and is specific for each acid, acetic having only 10-20 per cent the toxicity of benzoic."

Johannessohn,[7] in 1912, published another report, in which he made similar experiments as Bial with a series of formic acid, and came to the conclusion, that with acids the main part of the acid action is played by the undissociated molecules, not by the ions; and besides the larger the molecular weight the more pronounced is the bactericidal action.

Michaelis,[5] however, gave an account on the same subject in 1914, and agreed with Bial, saying: "Da sich herausgestellt hat, dass alle chemischen und biologischen Vorgänge, die von der Acidität der Lösung beeinflusst werden, nur die aktuelle Acidität (d.h. die durch die Wasserstoffzahl definierte Acidität) massgebend ist, so erhellt die überwiegende Bedeutung derselben."

Against the results obtained by Michaelis, Traube,[9] 1921, published another opinion. He pointed out that some of the measures employed by Michaelis should be discarded and concluded that the acid action must be reduced rather to anion and undissociated molecules than to hydrogen-ion concentration, and further in biologic respects he concluded as follows: "Nach meiner Ansicht ist es viel richtiger, wieder die alte Titrationsacidität und Titrationsalkalität mehr in den Vordergrund der biologischen Betrachtungen zu stellen."

From physicochemical points of view, some authors attempt to compare the relationship between acid action and bacteria with surface tension, absorption, swelling and flocking character, osmotic action and electric potential of materials to be brought in contact,[10, 11, 12] but these can give no satisfactory explanation of the complicated processes in this problem. Such reactions as the coagulation of casein and swelling of fibrin, can at any rate, be taken as phenomena occurring in bacteria, which are composed at least of protein substances numbering 2,432, 902,008, 176,640,000 components [13]

Furthermore, if other circumstances such as utilization of various organic acids by bacteria as the source of carbon are taken into account,[14, 15] it may be reasonable to think that at the same optimum hydrogen-ion concentration of the solution, some different results may be obtained according to the kinds of acid taken in adjusting the reaction of culture media. This fact must especially be taken into account, inasmuch as the death-point of bacteria, i.e., the so-called physiologic constant is mostly subject to the chemicals employed, and decided differences may result from the composition of the fluid.

These are the cardinal points, which have led me to attempt the following experiments.

EXPERIMENTAL

I used a strain of B. coli (C_{26}), which was employed throughout the entire course of my experiments. The standard culture media were peptone (Witte) solutions, thus prepared: The peptone was dissolved in aqua distillata to 1.0 per cent. No salt was added to the media.

The amount of acid given in these tables is always the quantity of total acid contained in 100 c.c. of the 1.0 per cent peptone solution, after addition of one-fifth normal solution of the former. The number of bacteria was calculated from colonies obtained from 1.0 c.c. of culture fluid.

The death-point shows striking differences according to the kinds of acid employed, whether the acidity be determined by the titration method or electrometrically.

If we compare the difference of the death-point in each acid with that of the dissociation-constant and molecular weight of respective acids, no parallelism between these can be traced; they behave entirely independent of each other.

TABLE III

SULPHURIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.2	4.46	0		0		0	
2	3.7	4.64	2,940		54,000		5,076,000	4.63
3	3.4	4.72	1,380		14,000		1,512,000	4.72
4	3.2	4.87	3,800		117,000	4.88	25,800,000	5.18
5	2.7	5.37	204,000	5.29	18,600,000	5.40	48,600,000	5.51
6	2.0	5.76	1,728,000	5.70	19,800,000	6.00	78,000,000	6.30
7	1.5	6.50	1,458,000	6.43	25,200,000	6.73	90,000,000	6.78
8	1.0	6.85	7,560,000	6.80	60,000,000	6.93	104,000,000	7.02

TABLE IV

NITRIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.2	4.54	0		0		0	
2	3.7	4.63	68		21		0	
3	3.4	4.90	1,080		78,000	4.94	23,400	5.00
4	3.2	5.01	4,560		2,640,000	5.11	16,200,000	5.29
5	2.7	5.23	6,000	5.29	6,600,000	5.30	43,200,000	5.33
6	2.0	5.92	558,000	5.60	27,600,000	5.87	146,000,000	7.20
7	1.5	6.46	4,334,000	6.40	52,800,000	6.43	180,000,000	7.21
8	1.0	6.85	3,600,000	6.70	156,000,000	6.70	171,000,000	7.32

TABLE V

PHOSPHORIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	8.5	4.32	79		0		0	
2	8.0	4.43	660		345	4.48	1,354	4.52
3	7.6	4.48	900	4.50	1,440	4.51	4,600,000	4.69
4	7.2	4.55	1,440		1,500	4.57	18,600,000	4.71
5	6.8	4.76	8,400		25,400	4.76	39,000,000	
6	6.4	4.91	15,300		100,000	4.96	43,800,000	
7	5.4	5.00	78,000		6,600,000	5.00	110,000,000	5.75
8	4.3	5.44	4,200,000		19,200,000	5.58	121,000,000	6.09
9	3.1	6.01	21,000,000	6.07	30,000,000	5.95	164,000,000	6.40
10	1.5	6.63	22,200,000		54,000,000	6.61	150,000,000	7.19

TABLE VI
FORMIC ACID

INCUBA-	0		12		24		48	
TION TIME IN HOURS	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	2.7	5.40	0	5.40	0	5.41	0	5.41
2	2.3	5.63	2,701	5.54	20,000	5.78	4,260,000	5.82
3	2.0	5.80	3,480	5.80	100,000	6.00	33,000,000	7.06
4	1.75	6.02	38,880	6.01	7,200,000	6.28	72,000,000	7.19
5	1.50	6.32	168,200	6.31	43,200,000	6.45	102,000,000	7.22
6	1.25	6.49	859,000	6.50	50,600,000	6.67	138,000,000	7.30
7	1.00	6.80	798,000	6.77	60,000,000	6.92	108,000,000	7.29

TABLE VII
ACETIC ACID

INCUBA-	0		12		24		48	
TION TIME IN HOURS	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	3.7	4.87	11		2		0	
2	3.5	5.00	200		10	5.01	0	
3	3.3	5.18	1,380		44	5.20	1	
4	2.6	5.30	3,900		1,320		208	
5	2.4	5.45	3,910		630,000		25,200,000	5.48
6	2.2	5.85	3,720	5.86	818,000		39,000,000	
7	2.0	6.00	17,000		16,200,000	6.21	102,000,000	6.20
8	1.8	6.18	114,000		42,000,000		99,300,000	6.60
9	1.6	6.34	2,700,000		66,000,000	6.61	108,000,000	6.61
10	1.4	6.65	2,430,000		54,130,000		102,900,000	6.81
11	1.0	6.82	9,000,000		84,000,000	6.84	104,000,000	7.04

TABLE VIII
CHLORACETIC ACID

INCUBA-	0		12		24		48	
TION TIME IN HOURS	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	2.0	5.52	42		0		0	5.54
2	1.8	5.74	55		0		0	
3	1.65	5.90	200		19		0	
4	1.50	6.09	35		60		0	6.10
5	1.35	6.24	1,000		2,380,000	6.18	41,400,000	6.40
6	1.20	6.47	110,000		18,600,000	6.40	156,000,000	6.61

TABLE IX
PROPIONIC ACID

INCUBA-	0		12		24		48	
TION TIME IN HOURS	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	12.0	4.43	8		4	4.43	0	4.44
2	10.0	4.51	10		0	4.50	0	4.53
3	8.0	4.65	7		0	4.67	0	4.71
4	6.0	4.79	10		0	4.75	0	4.82
5	5.0	5.09	5		34		0	5.12
6	4.0	5.35	75		121		6	5.40
7	3.5	5.51	420		23,900		1,700	5.52
8	3.0	5.60	12,700		1,010,000	5.54	12,000,000	5.62
9	2.5	6.10	66,000		15,000,000		60,000,000	6.60
10	2.0	6.60	576,000	6.32	36,700,000	6.67	78,000,000	6.75
11	1.5	6.73	954,000	6.71	72,000,000	6.70	126,000,000	6.98

TABLE X—LACTIC ACID (OPTICALLY INACTIVE)

INCUBA-TION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.5	4.20	19		0		0	4.21
2	4.3	4.45	4	4.46	1	4.46	0	
3	4.0	4.57	1	4.56	0		9	4.59
4	3.8	4.61	1,320		1,223		2,216,500	4.62
5	3.5	4.70	1,380		3,000		2,484,000	4.80
6	3.2	4.91	2,000		500,000		13,800,000	4.94
7	2.7	4.93	4,400		1,200,000		18,000,000	5.60
8	2.0	5.50	5,700		8,000,000		60,000,000	6.01
9	1.7	5.84	380,000	5.80	9,000,000	5.97	62,000,000	6.23
10	1.5	6.18	440,000		15,000,000	6.30	99,000,000	6.34
11	1.5	6.24	1,380,000	6.26	61,000,000	6.26	114,000,000	6.72

TABLE XI—OXALIC ACID

INCUBA-TION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	2.2	5.67	0		0	5.67	0	5.66
2	2.0	5.86	0		0	5.87	0	5.87
3	1.8	5.93	1		0	5.98	0	5.99
4	1.6	6.20	0		0	6.30	2	6.30
5	1.4	6.39	600		132,000	6.41	45,000,000	6.46
6	1.2	6.61	8,100		41,400,000	6.70	96,000,000	6.72

TABLE XII—NORMAL SUCCINIC ACID

INCUBA-TION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	14.0	4.24	0		0	4.24	0	4.24
2	12.0	4.35	184		140	4.39	17	4.40
3	10.0	4.40	3,240		18,000	4.43	6,000,000	4.49
4	7.0	4.50	213,000		990,000	4.52	13,000,000	4.59
5	5.0	4.70	370,000		5,400,000	4.75	44,400,000	5.38
6	4.0	5.10	2,400,000	5.01	12,000,000	4.80	45,000,000	5.72
7	3.0	5.32	5,000,000		24,600,000	5.39	66,000,000	6.12
8	2.5	5.82	10,200,000		30,000,000	5.94	96,000,000	6.39
9	2.0	6.30	16,270,000		48,000,000	6.45	114,000,000	6.80
10	1.8	6.61	27,000,000	6.54	78,000,000	6.74	114,000,000	6.97
11	1.6	6.73	28,000,000	6.60	84,000,000	6.80	124,000,000	6.99
12	1.5	6.84	26,000,000	6.75	83,000,000	6.80	109,000,000	7.07

TABLE XIII—DEXTROTARTARIC ACID

INCUBA-TION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	7.5	4.20	11		0		0	
2	5.0	4.45	100		1,260		210,000	4.48
3	4.25	4.60	300		8,049		21,310,000	4.62
4	3.75	4.80	6,400				33,600,000	
5	3.0	5.00	3,900				37,000,000	
6	2.75	5.20	12,600		18,600,000		55,000,000	5.98
7	2.30	5.35	630,000		47,400,000		90,000,000	5.98
8	2.0	5.71	770,000	5.83	57,000,000	6.01	148,000,000	6.33
9	1.5	6.00	19,800,000	6.01	72,200,000		142,000,000	
10	1.0	6.20	30,600,000	6.12	70,000,000		130,000,000	6.75

TABLE XIV

CITRIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	9.0	4.40	1,320		99	4.40	0	
2	6.0	4.59	2,200		13,140	4.59	21,000,000	4.61
3	5.0	4.81	4,400		550,000		38,400,000	
4	4.0	4.99	4,200		354,000	5.03	37,000,000	5.12
5	3.0	5.16	11,000	5.19	27,900,000		39,000,000	5.39
6	2.0	6.20	41,000	6 16	28,000,000	6.31	66,000,000	6.72

The results of Experiment II are summarized in Table XV.

TABLE XV (I)

	ACID PER 100 C.C. OF MEDIA	P_H	P_H (AVERAGE)
1. Hydrochloric	4.2- 3.7 (c.c.)	4.51-4.70	4.6
2. Sulphuric	4.2- 3.7	4.46-4.63	4.6
3. Nitric	3.7- 3.4	4.63-4.90	4.8
4. Phosphoric	8.5- 8.0	4.32-4.52	4.5
5. Formic	2.7- 2.3	5.41-4.80	5.6
6. Acetic	3.3- 2.6	5.20-5.48	5.3
7. Monochlor	1.5- 1.35	6.10-6.40	6.3
8. Propionic	4.0- 3.0	5.40-5.62	5.5
9. Norm. Lactic	4.0- 3.8	4.57-4.61	4.6
10. Oxalic	1.6- 1.4	6.20-6.40	6.3
11. Norm. Succinic	12.0-10.10	4.35-4.49	4.4
12. Norm. Tartaric	7.5- 5.0	4.20-4.45	4.4
13. Citric	9.0- 5.0	4.49-4.60	4.5

TABLE XV (II)

ACIDS	DISSOCIATION-CONSTANT (100 k)	MOLECULAR WEIGHT
1. Chloracetic	0.16	94.484
2. Oxalic	4.0	126.048
3. Formic	0.021	46.016
4. Acetic	0.0018	60.032
5. Nitric		63.018
6. Propionic	0.0014	74.048
7. Lactic	0.014	90.048
8. Hydrochloric		36.468
9. Sulphuric		98.086
10. Tartaric	0.097	150.048
11. Citric	0.083	210.08
12. Phosphoric		98.086
13. Succinic	0.0066	118.048

EXPERIMENT III

In the foregoing experiment, I observed that a certain acceleration or delay in development of bacteria may be induced at a low concentration of different acids, so I attempted here to go into detail in this respect; though for this purpose a large number of controls is required, I adopted the following two methods, to obtain a general consideration on this subject.

Method A.—To ninety parts of peptone solution ten parts of buffer mixture (boric acid, KCl and NaOH, after Clark, $P_H = 8.4$)[17] were added to give the solution $P_H = 8.3$. The same series of acids as employed in Experi-

ment II were added in one-fifth normal solution to the above mixture, thus the whole solution attained about $P_H = 7.0$. Here the amount of acids to give final—P_H value differs widely according to their dissociation-constants. Thus we may assume that these solutions consist of exactly the same components, except in their amount of anion (Table XVI).

Method B.—Equal quantities (2.0 c.c.) of one-fifth normal solutions of each acid are added to the peptone water. These mixtures receive further 20.0 c.c. of phosphate solution which is prepared with proper amounts of one-fifth mol solutions of primary and secondary sodium phosphate in various

	ACID	Na$_2$HPO$_4$2H$_2$O	NaH$_2$PO$_4$	2% PEPTONE	AQ. DEST.
1. Hydrochloric	2.0 c.c.	12.75 c.c.	7.25 c.c.	50.0 c.c.	28.0 c.c.
2. Sulphuric	"	13.00	7.00	"	"
3. Nitric	"	12.20	7.80	"	"
4. Phosphoric	"	15.64	4.36	"	"
5. Formic	"	12.00	8.00	"	"
6. Acetic	"	12.05	7.95	"	"
7. Propionic	"	13.50	6.50	"	"
8. Lactic	"	11.70	8.30	"	"
9. Oxalic	"	11.80	8.20	"	"
10. Succinic	"	13.00	7.00	"	"
11. Tartaric	"	12.10	7.90	"	"
12. Citric	"	11.90	8.10	"	"

20.00 c.c. in all.

TABLE XVI

INCUBATION TIME IN HOURS	18	24	36	48	AFTER TEN DAYS
ADJUSTED BY	NUMBER OF BACTERIA PER C C.				P_H
1. Hydrochloric	60,000,000	127,000,000	155,800,000	128,400,000	7.99
2. Sulphuric	48,780,000	70,000,000	96,600,000	114,000,000	8.08
3. Nitric	98,400,000	145,400,000	164,200,000	142,000,000	8.12
4. Phosphoric	51,300,000	63,000,000	96,000,000	113,000,000	7.59
5. Formic	39,000,000	48,180,000	72,000,000	72,600,000	8.37
6. Acetic	55,800,000	58,800,000	85,200,000	109,800,000	8.10
7. Propionic	37,200,000	63,000,000	91,000,000	92,400,000	7.97
8. N. Lactic	34,000,000	51,600,000	66,000,000	83,900,000	8.14
9. Oxalic	47,000,000	90,000,000	91,600,000	73,100,000	7.87
10. N. Succinic	44,400,000	62,000,000	87,000,000	104,400,000	8.19
11. N. Tartaric	63,600,000	78,000,000	114,000,000	63,000,000	8.20
12. Citric	46,800,000	69,600,000	84,000,000	64,800,000	7.88

TABLE XVII

INCUBATION TIME IN HOURS	18	24	48	72
ADJUSTED BY				
1. Hydrochloric	33,600,000	108,000,000	165,000,000	198,000,000
2. Sulphuric	25,000,000	78,000,000	79,000,000	96,000,000
3. Nitric	58,000,000	138,000,000	169,600,000	202,000,000
4. Phosphoric	49,000,000	60,000,000	72,000,000	88,000,000
5. Formic	19,200,000	54,000,000	76,800,000	118,000,000
6. Acetic	50,400,000	68,400,000	114,000,000	156,000,000
7. Propionic	21,000,000	76,800,000	96,000,000	99,000,000
8. N. Lactic	18,600,000	54.100,000	83,500,000	117,000,000
9. Oxa'ic	40,400,000	94,200,000	96,000,000	133,000,000
10. N. Succinic	25,000,000	87,600,000	98,000,000	103,000,000
11. N. Tartaric	28 200,000	54,000,000	106,000,000	149,000,000
12. Citric	27,600,000	66,000,000	68,000,000	126,000,000

proportions, to bring the reaction of the whole mixture to $P_H = 7.0$ (Table XVII).

Tables XVI and XVII lead us to the following conclusions. In these tables the bacteria are given in average numbers from repeated experiments, and Table XVI shows a remarkable difference in number of bacteria according to acids added to the culture fluid. The development of bacteria is accelerated by some inorganic acids, e.g., nitric and hydrochloric acid; among them the former has the most favorable effect upon bacterial development. On the contrary the growth of bacteria is remarkably retarded by addition of sulphuric and phosphoric acid. On the other hand no striking difference in action may be noticed among organic acids, the accelerating power also being much less than that of nitric or hydrochloric acid. Moreover the end-reaction of the fluid is in close relationship with the kinds of acid employed. The result of Table XVII coincides on the whole with that of Table XVI. The end-reaction suffers no remarkable change from the buffer action of a large quantity of phosphoric acid. Detailed descriptions are omitted here.

From these results it is obvious that nitric acid gives particularly good results as a growth-promoting agent, when it is added in proper amount to the media, at least this is true with colon bacilli, and hence nitric acid should be employed in adjusting the reaction of media for B. coli.

EXPERIMENT IV

It was already stated by Rosenblatt and Rozenband[18] in 1907 that acetic acid stands in the way of alcoholic fermentation. Dam[19] recognized the same fact with lactic acid. On the other hand, Clark, Wyeth and Scheer[20, 21, 22] gave some accounts of the effect of these acids upon the final H-ion concentration by the alcoholic fermentation of B. coli.

These latter authors are of the opinion, that, when such acids as may be produced in the course of fermentation already exist from the beginning of cultivation, the fermentation decreases considerably in consequence of the retarding action of acids added.

It seems incorrect to estimate the process of fermentation by way of determination of the end hydrogen-ion concentrations, as for instance, colon bacilli which may perish during the fermentation by the acids which they produced themselves. However, from facts mentioned above we must bear in mind, that besides H, another important factor in the acid-action should not be neglected. This is anion. It has led me to attempt again to examine the end-P_H of culture media, which have previously been adjusted to the P_H of 7.0 with the series of acids used in my foregoing experiments.

The media used for this purpose were prepared like those employed in Exp. III. B., to which glucose was added to give a 2.0 per cent solution.

The final P_H given in Table XVIII is that of five to seven days after the inoculation when no live cells in the media could be detected. The results thus obtained from my experiment show approximate agreement with those of Clark and others as to hydrochloric, acetic and lactic acid. However, not

TABLE XVIII

	FINAL P_H (5-7 DAYS)
1. Hydrochloric	4.56
2. Sulphuric	4.56
3. Nitric	4.63
4. Phosphoric	4.58
5. Formic	4.62
6. Acetic	4.63
7. Propionic	4.68
8. N. Lactic	4.62
9. Oxalic	4.56
10. N. Succinic	4.63
11. N. Tartaric	4.63
12. Citric	4.62

only these three kinds of acids but also another series of acids, as given in Table XVIII, may show the same.

The differences in the end-reaction of media differ somewhat widely in the reports of Clark and others. This is probably due to the difference in quantities of acid and buffer solution.

EXPERIMENT V

This experiment was made in order to know the results of neutralizing of media with equivalent quantities of NaOH to the bactericidal concentration of acids.

TABLE XIX

	P_H (INITIAL)	N.B. (1 DAY)	N.B. (2 DAYS)	P_H (10 DAYS)
1. Hydrochloric	7.40	101,000,000	90,000,000	7.91
2. Nitric	7.40	144,000,000	122,000,000	7.95
3. Tartaric	7.40	86,000,000	78,000,000	8.20
4. Acetic	7.23	96,000,000	90,000,000	8.00
5. Oxalic	7.41	64,000,000	90,000,000	8.01
6. Chloracetic	7.38	89,000,000	48,000,000	7.81

Peptone solutions are reduced to coagulation, which is more or less reversible by the addition of acid or alkali. About this fact Kligler[23] reports as follows: "It is a well-known fact that when media are neutralized to phenolphthalein, a precipitate is produced which may be partly redissolved by the addition of acid. If this precipitate is filtered off, the nutritive value of the medium is appreciably lowered." Among the kinds of acids employed in my experiments, oxalic acid was the one which caused the irreversible coagulation of peptone solution. After addition of the acid I added the same amounts of alkali (NaOH), but the precipitate was never redissolved. Notwithstanding this property, the nutritive value was never affected if no filtering operation was made such as Kligler performed.

EXPERIMENT VI

This experiment was attempted to gain some aspects about the results obtained with mixtures of equal parts of two different acids, which are quite different from each other in their bactericidal concentrations.

TABLE XX

(OXALIC + TARTARIC		P_H	N.B. (24 HRS.)			$\frac{N}{5}$ ACID (O.C.)	P_H (DEATH-POINT)
1	3.5 (c.c.)	4.70	0	(Oxal. Tart.)		2.9-2.5	5.2
2	3.0	5.10	0	Oxalic		1.6-1.4	6.3
3	2.9	5.20	1	Tartaric		7.5-5.0	4.4
4	2.7	5.23	11				
5	2.5	5.48	1,008,000				
6	2.0	6.0	18,080,000				
7	1.5	6.51	22,680,000				
8	1.0	6.95	34,800,000				

Oxalic and tartaric acid were used for this purpose.

	BY	DEATH-POINT
Oxalic acid	....	$P_H = 6.3$
Tartaric acid	....	$P_H = 4.4$
Acid mixture	....	$P_H = 5.3$

These results are brought out more clearly in Table XX. It is particularly interesting to note the approach of the death-point of both acids towards each other. This may be, in other words, a case of buffer-action of weak acid.

EXPERIMENT VII

Much study has been bestowed by many investigators on the effects of so-called neutral salts upon bacterial growth. Lately Sherman and Holm[24] published some notes thereon and said: "A strain of B. coli which showed no growth at P_H of 4.8 (adjusted by HCl) in 1.0 per cent peptone solution at 37° C., grew quite readily in the same medium to which NaCl had been added to make a 0.2 mol solution. On the other hand, Na-citrate shows no widening effect upon the P_H zone of growth and even narrows the limits of H-ion concentration at which this strain will grow."

At first, I doubted these widening effects of NaCl, which have been emphasized by these authors, thinking that the results may probably be due to the so-called "common-ion-effect," through which the ionization of the acid, here HCl, was affected and correspondingly caused the lowering of the H-ion concentrations. Secondly, I attempted to solve, just by means of this fact, whether the acids play a rôle on the bacteria as anion or as undissociated molecules.

As shown in Table XXII acetic acid clearly displays its bactericidal action as undissociated molecules.

As shown in Table XXI, the addition of NaCL to 0.1 mol causes apparently not only the shifting of limits of growth which were attained by the use of HCl, but also a decided acceleration in growth of bacteria, 0.3 mol NaCl being rather less effective. In this point Sherman is right. But there is no agreement of results obtained by Sherman and myself, when Na-citrate has been added to obtain 0.2 mol solution. In my case bacterial growth was observed after addition of Na-citrate, though it has no such accelerating effect as NaCl. It may, of course, be taken into consideration

TABLE XXI

CONTROL

	$\frac{N}{5}$ HCL	P_H	N.B. 48 HR.
1	4.8 (c.c.)	4.25	0
2	4.5	4.34	0
3	4.3	4.50	0
4	3.6	4.69	60,000

I

	$\frac{N}{5}$ HCL	NaCL	P_H	N.B. 24 HR.	48 HR.
1	4.8 (c.c.)	01. M	4.32	10,231,000	37,800,000
2	4.5	"	4.40	17,400,000	43,000,000
3	4.3	"	4.60	30,600,000	44,400,000
4	3.6	"	4.75	34,000,000	184,000,000

II

	$\frac{N}{5}$ HCL	NaCL	P_H	N.B. 24 HR.	48 HR.
1	4.8 (c.c.)	0.3 M.	4.33	268	300
2	4.5	"	4.42	227	480,000
3	4.3	"	4.64	203,000	3,060,000
4	3.6	"	4.78	34,800,000	162,000,000

III

	$\frac{N}{5}$ HCL	Na-CIT.	P_H	N.B. 24 HR.	48 HR.
1	4.8 (c.c.)	0.2 M.	6.94	33,000,000	30,000,000
2	4.5	"	7.02	36,000,000	30,000,000
3	4.3	"	7.10	51,000,000	–
4	3.6	"	7.25	58,000,000	–

TABLE XXII

	$\frac{N}{5}$ ACETIC	Na-ACET.	P_H	N.B. (48 HR.)
1	3.4 (c.c.)	–	5.03	0
2	"	0.01 Mol	5.53	0
3	"	0.05	5.81	40
4	"	0.1	6.12	0
5	"	0.2	6.36	600
6	"	0.3	6.49	6,340

TABLE XXIII

CONTROL

	$\frac{N}{5}$ CITRIC A.	P_H	COLONIES (24 HR.)
1	9.0 (c.c.)	4.41	3
2	8.0	4.48	2,520
3	6.0	4.60	130,000
4	5.0	5.01	4,860,000

	$\frac{N}{5}$ CITRIC A.	Na-CIT.	P_H	N.B. (24 HR.)
1	9.0 (c.c.)	0.1 M.	6.22	24,400,000
2	8.0	"	6.30	65,400,000
3	6.0	"	6.41	55,000,000
4	5.0	"	6.53	88,200,000

With citric acid its P_H+ may be of rather more importance, as shown in Table XXIII.

TABLE XXIV

CONTROL

	Na-FORMATE	P_H	N.B. (24 HR.)
1	0.01 (M.)	7.50	30,000,000
2	0.05	7.59	33,000,000
3	0.1	7.68	27,000,000
4	0.2	7.71	25,200,000
5	0.3	7.75	16,200,000
6	0.4	7.81	8,580,000
7	0.5	7.89	960,000

	$\frac{N}{5}$ FORMIC A.	Na-FORMATE	P_H	N.B. (24 HR.)
1	2 7 (c.c.)	~	5.42	11
2	"	0.01 (M.)	5.51	0
3	"	0.05	5.59	0
4	"	0.1	5.72	4
5	"	0.2	5.90	14
6	"	0.3	6.11	127
7	"	0.4	6.16	67
8	"	0.5	6.21	46

It is clear from this Table XXIV, that formic acid also displays its toxic action upon bacteria as undissociated molecules.

TABLE XXV

CONTROL

	Na-OXALATE	P_H	N.B. (24 HR.)
1	0.001 (M.)	7.31	163,800,000
2	0.005	7.52	101,400,000
3	0.01	7 62	182
4	0.05	7.99	44,400,000
5	0.1	8.37	6,800,000
6	0.2	8.89	0

	$\frac{N}{5}$ OXALIC A.	Na-OXALATE	P_H	N.B. (24 HR.)
1	2.0 (c.c.)	~	6.24	0
2	"	0.01 M.	6.75	4
3	"	0.05	7.31	42,300,000
4	"	0 1	7.68	16,200,000

that a simultaneous lowering of H-ion concentration of media, owing to the slight alkalinity of Na-citrate solutions, may occur.

As may be deducted from the results in Table XXV, Na-oxalate acts upon bacterial growth in quite a different way, as compared with other sodium salts of various acids. While smaller amounts of these salts display strong toxicity, larger amounts show rather less toxic power, but further increase of the salts inhibits the growth of bacteria again. Some notes about these facts may shortly be given as follows:

1. When these salts are used in very small amounts, the amount of oxalic-ion too will be small, consequently they can display almost no toxic effects.

2. A moderate amount of this salt will reduce a moderate quantity of oxalic-ion, so it may act much more toxic accordingly.

3. Further increase in the amount of salts may cause the decrease of toxicity because of the strong increase in alkalinity, the dissociation of salt-

molecules might be depressed by the presence of anion of some other electrolytes.

4. The total inhibition of bacterial growth by the larger amounts of these salts is apparently due to the strong alkalinity.

SUMMARY AND CONCLUSIONS

From the foregoing experiments we may summarize the results as follows:

1. The nature of acidification of media at a certain phase should not always be ascribed to the existence of any sugars in the media. Most probably it comes from the decomposition of protein substances by bacteria.

2. The final H-ion concentrations show remarkable differences according to the kinds of acids.

That is to say, they differ in death-point (Table XV, Table XVIII) and in degree of alkalization when acids were added in small amounts. Furthermore, the bacterial growth never runs parallel with this degree of alkalization of media (Tables XVI, XIX). Accordingly, we wish rather to emphasize the difference in value of acids as a buffer. Formic acid is most liable to alkalization, phosphoric and oxalic acids least. With lactic acid the result of my experiment differs from that obtained by Koser.

3. Many acids vary in their accelerating or retarding effect on bacterial growth, when added in low concentrations. With inorganic acids, the presence of nitric as well as hydrochloric acid stimulates the growth of bacteria, while sulphuric and phosphoric acids effect delay in growth. On the other hand, organic acids have, as a whole, no stimulating effect, except acetic and oxalic acid which, however, act far inferiorly to nitric or hydrochloric acid. So it appears quite correct, at least with colon bacilli, that culture media should be adjusted with nitric acid, should any acidifying be necessary.

4. With media which contain sugars there exists a certain relationship between the final reaction and the kind of acids employed for adjustment of initial reaction. Now we may understand much about the conditions which induce a different final P_H when different sugars are used.

5. The nutrient value of peptone is scarcely altered by the coagulating action of acid or alkali.

6. The effect of acid upon microorganisms should not only be attributed to its H-ion concentrations but also to its anion and further to its undissociated molecules, as stated already by Krönig and Paul.

Furthermore, it should be remembered that the acid-action upon microorganisms is quite specific; even one and the same acid may act differently on different microorganisms.

To sum up, in biologic researches, in which any culture of microorganisms is made and the optimum H-ion concentration or death-point of bacteria is discussed, we must always, besides CH+, give some account in detail as to the kinds of acid or alkali and their concentration used (in other words normality).

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BIBLIOGRAPHY

[1] Kojima, S.: Japanese Jour. of Exp. Med., 1923, vii, No. 11 and 12, p. 1029.
[2] Conradi, H. and Bierast, W.: Kolle u. Wassermann, 1913, vi, 488.
[3] Burow, E.: Centralbl. f. Bakt., 1921, I Abt. (orig.) lxxxvi, 517.
[4] Krönig, B. and Paul, T.: Ztschr. f. Hyg., 1897, xxv, 1.
[5] Bial, M.: Ztschr. f. Physik. Chem., 1902, xl, 513.
[6] Winslow, C. E. A.,   T. E.:   Jour. Infect. Dis., 1906, iii, 541.
[7] Johannessolin, F.:   ii, 97.
[8] Michaelis, L.: Die   1914.
[9] Traube, J.: Biochem. Ztschr., 1921, cxx, 108.
[10] Somogyi, R.: Biochem. Ztschr., 1921, cxx, 103.
[11] Somogyi, R.: Biochem. Ztschr., 1921, cxx, 100.
[12] Traube, J. and Somogyi, R.: Biochem. Ztschr., 1921, cxx, 91.
[13] Wells, H. G.: Chemical Pathology, Ed. 4, p. 171.
[14] Braun, H., and Cahn-Bronner, C. E.: Biochem. Ztschr., 1922, cxxx, 226 and 276.
[15] Koser, S. A.: Jour. Bacteriol., 1923, viii, No. 5, p. 493.
[16] Smith, G.: Jour. Hyg., 1920, xix, 133.
[17] Clark, W. M.: The Determination of Hydrogen Ions, 1922.
[18] Rosenblatt and Rozenband: Compt. rend. d' Acad. d. Sc., 1907, clxix, 309.
[19] Dam, W. van.: Biochem. Ztschr., 1918, lxxxvii, 107.
[20] Clark, W. M.: Jour. Biol. Chem., 1915, xxii, 87.
[21] Wyeth, T. J. S.: Biochem. Jour., 1919, xiii, 382.
[22] Scheer, K.: Biochem. Ztschr., 1922, cxxx, 535 and 545.
[23] Kligler, I. J.: Jour. Bacteriol., 1917, ii, 351.
[24] Sherman, J. M., and Holm, G. E.: Jour. Bacteriol., 1922, vii, No. 5, p. 465.

KOLMER'S CHOLESTERINIZED VERSUS ACETONE INSOLUBLE ANTIGEN*

By Wilfred H. Kellogg, M.D., Berkeley, California

A PREVIOUS study of the Kolmer modification of the Wassermann test, carried out particularly with a view of clearing up certain discrepancies between laboratories in their reports on the same specimens, which discrepancies seemed at times to be related to differences in the antigen, has been published.†

At the present time I want to report on a further series of comparative tests totaling nearly 10,000. In the former article results of a series of 506 consecutive blood specimens tested by the Kolmer method using Kolmer's antigen, and by the Kolmer method using acetone insoluble antigen, and also tested by another method using the same two antigens, were reported. This made four tests in all for each serum. Some of the results are briefly summarized as follows:

Nine serums were markedly stronger in the Kolmer test with acetone insoluble antigen than they were in the same test with Kolmer's antigen, and the same serums gave a stronger reaction in the other test with the acetone insoluble antigen than they did with the Kolmer showing that the difference was in the antigen and in favor of the acetone insoluble type.

Seventeen serums were just the opposite; that is, they gave stronger reactions in both types of Wassermann test with the Kolmer antigen than they did in the same test with the acetone insoluble antigen, again demonstrating the difference to be the antigen but this time in favor of Kolmer's cholesterinized antigen.

Of the above tests two were 4-plus in Kolmer's test and negative in the same test using the other antigen, while three were 4-plus in the Kolmer test using acetone insoluble antigen and only one plus when Kolmer's antigen was used. One was 3-plus with the acetone insoluble and negative with the Kolmer antigen, in both instances the Kolmer method being used. Table I shows that the advantage is with the cholesterinized antigen, excepting that there are a few more 4-plus reactions with the acetone insoluble than with the cholesterinized antigen.

Acetone Insoluble Antigen.

Kolmer Antigen	−	+	++	+++	++++
−		45	5	1	
+	113		8	5	1
++	26	61		7	1
+++	12	62	65		7
++++		20	34	84	

Fig. 1.—Each individual serum of the number shown in any square gave the result shown at the top of the column for the acetone insoluble antigen and at the left with the Kolmer antigen. Consequently, all serums placed above the diagonal line were stronger with the acetone insoluble antigen and those below the line were stronger with the Kolmer antigen.

TABLE I

DEGREE OF REACTION	KOLMER TEST		OTHER TEST	
	WITH KOLMER ANTIGEN	WITH ACETONE INSOLUBLE ANTIGEN	WITH KOLMER ANTIGEN	WITH ACETONE INSOLUBLE ANTIGEN
−	10	32	11	20
+	19	13	24	14
++	15	8	12	5
+++	20	9	25	13
++++	26	28	15	36
Anticomplementary	0	0	3	2

Based on the above observations the conclusion was reached that variation in antigen is the greatest single cause for disagreement between laboratories, and that there are some syphilitic serums giving a positive reaction with cholesterinized antigen and a negative or a weaker reaction with the acetone insoluble type and vice versa.

At this time, as a result of the testing of a further series of 9307 specimens, the conclusion is reached that the difference in reaction with the two types of antigen as above noted is not so important, as indicated by the results obtained in the smaller series of tests. In the present series of 9307 specimens, all were tested twice by the Kolmer method, one test with Kolmer's antigen and the other with acetone insoluble antigen. There were 2023 serums giving a positive reaction of greater or lesser degree in one or both tests. Conflicting results, principally as to the degree of reaction were obtained with 557 of this number. The diagram (Fig. 1) is arranged to show these differences graphically.

It will be noted that among the serums that were negative with Kolmer's cholesterinized antigen, there were 45 that were 1-plus, 5 that were 2-plus, one that was 3-plus and none that were 4-plus with the acetone insoluble antigen. Of those that were negative with the acetone insoluble antigen 113 were 1-plus, 26 were 2-plus, 12 were 3-plus, and none were 4-plus with the Kolmer antigen. It is concluded, therefore, as a result of this more extended series of observations that, while the difference in reaction of different serums to the two types of antigen is definitely demonstrated, it is not the important factor that seemed likely from the first series of tests. The use of two antigens and the double testing of serums is not demonstrated as a necessity but one source of conflicting reports from different laboratories is clearly shown and should be borne in mind by clinicians. The greater sensitivity as compared to the acetone insoluble type, of the cholesterinized antigen, in the form used by Kolmer, is shown.

THE PREPARATION AND CLINICAL TESTS OF AN ANTISTREPTO-COCCUS (SCARLATINAE) SERUM*

By N. S. Ferry, Ph.B., M.D., R. W. Pryer, M.S., Dr.P.H., and L. W. Fisher, B.S., Detroit, Michigan

IN January, 1924, Dochez[1] described an antitoxic serum, for the treatment of scarlet fever, which was tested out clinically and reported on by Blake, Trask and Lynch.[2] This serum was obtained from a horse immunized to the Streptococcus scarlatinae by a special method said by Zingher[3] to be carried out by injecting into the cellular tissues of the neck of the horse a small amount of liquefied agar and into this nodule of agar, after it has solidified, the sedimented live organisms. The bacteria, thus protected from the injurious phagocytic action of the leucocytes of the blood of the animal, are supposed to produce a toxin which, in turn, stimulates the formation of antibodies. According to Zingher, "One of the objectionable features of this method of animal inoculation is that a large sloughing ulcer is produced at the site of the injected mass of agar, which is discharged as a foreign body." Blake and his associates described the

CASE 11932.—Age nine, female; admitted June 23, 1924. Onset June 21. Remarks: Heavy rash. Quite sick. June 23 received 30 c.c. serum. Results: Temperature normal on second day.

CASE 11934.—Age seven, male; admitted June 23, 1924. Onset June 21. Remarks: Quite sick. Faint rash. Temp. 104°. June 23 received 30 c.c. serum. Results: Temperature from 104° to 99.2° next day.

CASE 11752.—Age seven, male; admitted May 11, 1924. Onset May 9. Remarks: Acutely ill. Heavy rash. May 12, received 15 c.c. serum. Results: Temperature May 11, 103.5°; May 13, 99.8°; May 14, normal.

CASE 11758.—Age twenty-six, female; admitted May 14, 1924. Onset May 12. Remarks: Acutely ill. Heavy rash. May 15, received 30 c.c. serum. Results: Temperature 102.4° on May 14th to normal May 16th.

CASE 11696.—Age thirty, male; admitted May 2, 1924. Onset April 29. Remarks: Rash covers entire body. Throat red and swollen. May 3, received 25 c.c. serum. Results: Temperature dropped from 102° to 99° in 24 hr.

CASE 11959.—Age twenty-three, female, admitted July 6, 1924. Onset July 4. Remarks: Heavy rash. Temp. 104° July 7, received 30 c.c. serum. Results: Temperature July 7, 104°; July 8, 102.2°; July 9, 100.2°; July 10, normal.

In order to increase the potency of this serum and at the same time eliminate as far as possible urticarial rashes which usually follow in a certain proportion of cases injected with horse serum, it has been concentrated by the precipitation of the globulins according to a method somewhat similar to the preparation of other well-known concentrated serums. Agglutination tests show that this concentration has increased the titer of the serum about six times. While this does not necessarily imply that its protective value has increased to that extent, it is, more or less, an index of the concentration of the antibodies present. Taking into consideration the results usually following the concentration of antidiphtheritic and antitetanic serum, this increase in antibody content is also about what should be expected. So far the few cases upon which this concentrated serum has been tested have given extremely favorable results with 10 c.c. doses. These cases will be reported later.

CONCLUSIONS

1. An antistreptococcus scarlatinae serum has been prepared with both antitoxic and antibacterial properties.

2. This serum has proved of value as a therapeutic agent in scarlet fever.

3. The serum has been concentrated to at least six times its original strength with favorable clinical results.

REFERENCES

[1]Dochez, A. R., and Sherman, Lillian: Studies Concerning the Significance of Streptococcus Hemolyticus in Scarlet Fever, Proc. Soc. Exper. Biol. and Med., 1924, xxi, 194.
[2]Blake, F. G., Trask, J. D., and Lynch, J. F.: Treatment of Scarlet Fever with Scarlatinal Antistreptococcic Serum, Jour. Am. Med. Assn., 1924, lxxxii, 712.
[3]Zingher, A.: The Dick Test in Normal Persons and in Acute and Convalescent Cases of Scarlet Fever, Jour. Am. Med. Assn., 1924, lxxxiii, 432.
[4]Dick, G. F., and Dick, Gladys H.: A Scarlet Fever Antitoxin, Jour. Am. Med. Assn., 1924, lxxxii, 1246.
[5]Schultz, W., and Charlton, W.: Serologische Beobachtungen am Scharlachexanthem, Ztschr. f. Kinderh., Berl., 1918, Orig., xvii, 328.

THE EFFECT OF CONSTIPATION AND CATHARSIS ON UROBILINURIA*

By R. S. Dean, M.D., Cleveland, Ohio

EXAMINATION of the urine for urobilin has never been useful in the clinic, partly because of the obscure nature of this neglected pigment, and also because of the enormous variation in amount shown by individual cases. The influence of constipation and catharsis upon the variation in individual cases is the theme of this study. It is hoped that this and similar studies may throw some light on the physiology and pathology of urobilin and urobilinogen—for, unless otherwise stated, these two substances are here studied as one—and thus remotely come to bear on the problems of liver function and hemoglobin. This particular study certainly bears directly on the question of absorption of bile and stercobilin from the intestine, which has recently been reopened by Whipple,[1] who with good evidence asserts that no such absorption occurs. Through an understanding of the effect which constipation and catharsis have on the excretion of urobilin, much may perhaps be learned of the distribution and production of this obscure pigment.

That constipation and catharsis influence the output of urobilin in the urine was observed by several of the earlier writers on this subject. Special mention should be made of the work of Gerhardt[2] and Hildebrant.[3] Most of the early work was done without quantitative observations, but many of the recent investigators have ignored the influence of such simple factors on urobilinuria.[4, 5, 6]

METHOD

The methods used for the present work are those outlined by Wilbur and Addis,[4] with the following modifications:

Instead of grinding the feces in a mortar, the twenty-four hour specimen was placed in an ordinary three quart glass butter churn, which was attached to a 1/40 H.P. electric motor. Water was added to make a volume of 2000 c.c. This quantity of water was used because it was found that a fine, even emulsion could thus be more easily made, and the number 2000 made calculations easy. The hand spectroscope used by Wilbur and Addis was discarded in favor of the usual three-tube laboratory spectroscope (Bunsen and Kirchoff model), which showed the absorption bands at a higher dilution and consequently gave a more exact endpoint. The Wilbur and Addis calculations are based on the number of dilutions required to obliterate the urobilin spectroscopic band, plus the number of dilutions required to obliterate the urobilinogen spectroscopic band. The urobilin band practically always disappears last when feces are examined; and the urobilinogen band always disappears last when urine from patients with pathologic urobilinuria is examined. In the present study calculations have been made from the number of dilutions required to obliterate both spectroscopic bands. This modification was used by Blankenhorne[1] while studying the

CASE V.—Advanced Hodgkin's disease. Pronounced involvement of all the external lymph nodes was present. The afternoon temperature was usually near 39.5°. At a later date postmortem examination showed also extensive involvement of the liver. Graph 5 shows an increase of urobilinuria on the third day of constipation. Daily bowel movements without catharsis on the two following days are accompanied by a decrease in urobilinuria, with no definite change in the temperature curve.

CASE VI.—Lobar pneumonia. The patient was admitted after he had been ill one week. The entire left lung was consolidated. Graph 6 shows urobilinuria, which is increased by constipation, abolished by catharsis, and reappears with subsequent constipation. The patient was febrile on the first, second and third days. The temperature was normal on the fifth, sixth and seventh days. On the eighth day the temperature was 38°, and on the ninth day, 36.5°. Thereafter the temperature was normal. Besides the effect of catharsis and constipation, the temperature appears to affect urobilinuria. This is shown most distinctly on the eighth, ninth and tenth days.

Graph 7.   Graph 8.   Graph 9.
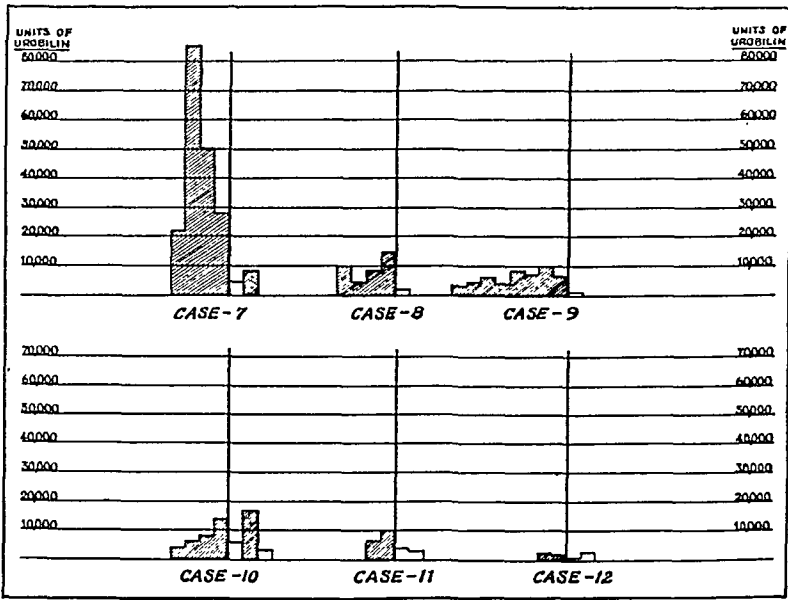
Graph 10.   Graph 11.   Graph 12.

Chart 2.

CASE VII.—Lobar pneumonia. The patient was admitted on the second day of his illness. The right lower lobe was consolidated. There was no bowel movement on the second day of his illness, and this study was begun on the third day. On this, the third day of constipation, Graph 7 shows a very large increase of urobilinuria, followed by a decrease on the fifth and sixth days. Catharsis given on the seventh day is accompanied by a further decrease. On the eighth day there was no bowel movement and the urobilinuria again increases. The temperature changes may exp'ain the decrease without catharsis on the fifth and sixth days, as there was a definite fall of temperature on the fifth day and a further fall accompanying the crisis on the sixth day.

CASE VIII.—Pseudoleucemia. The clinical diagnosis was confirmed by autopsy. The chief clinical findings were profound anemia, general adenopathy, a large spleen, and slight enlargement of the liver. There was an irregular fever. Graph 8 shows some irregularity of output regardless of catharsis and constipation. It should be mentioned that on the

first day of constipation the fever was higher than on any other day represented in the graph. Catharsis on the fifth day is accompanied by a marked decrease in urobilinuria.

CASE IX.—Tuberculous peritonitis. There was an irregular fever. With daily bowel movements there was a low grade urobilinuria. Constipation of eight days' duration was induced by giving paregoric. Urobilinuria was definitely increased by this prolonged constipation, but the increase was small and irregular as compared with that in cases showing a greater output of urobilin in the urine with daily defecation. Catharsis on the ninth day almost freed the urine of urobilin. The study was repeated in the same patient with similar results.

CASE X.—Pulmonary tuberculosis. The onset of the disease was acute. The temperature was continuously near 39°. Graph 10 shows an increase of urobilinuria accompanying four days of constipation. On the fifth day there is a decrease accompanying defecation without catharsis. On the sixth day with no bowel movement urobilinuria again increases to its highest level represented in the graph. On the seventh day it again falls accompanied by defecation. It is possible that the large amount of urobilin in the urine on the sixth day was due in part to absorption from contents in the intestinal tract which had collected on the days of constipation, and were only partly evacuated on the fifth.

CASE XI.—Pernicious anemia. The anemia was severe with a regular afternoon rise of temperature to 39°. The study of urobilinuria was not begun until after the patient had been constipated for three days. Graph 11 shows a moderate degree of urobilinuria reached after five days of constipation, and on the sixth day it is reduced to a very low level by moderate catharsis.

CASE XII.—Anemia of unknown origin. There were regular daily defecations with slight urobilinuria. Graph 12 shows a slight but definite reduction of urobilinuria caused by catharsis. On the day following the catharsis, there was one bowel movement, and the urobilinuria resumes, approximately, the same level as on the days preceding catharsis.

DISCUSSION

Besides the cases presented, others were studied. They similarly showed an increase in urobilinuria during constipation and a decrease with catharsis. Among them was a case of cirrhosis of the liver, but in this instance on repeated trials the increase and decrease of urobilinuria were not always present. This lack of constancy may have been due to irregularity of absorption from the intestine. That intestinal absorption was interfered with in this patient was shown by the repeated recurrence of ascites after tapping. This case is mentioned as the only one in which there was found any departure from the rule that constipation increases urobilinuria and catharsis decreases it.

In lobar pneumonia it must be remembered that there is a period of maximum urobilinuria in the course of the disease.[a] The increase and decrease might be confused with changes associated with constipation and catharsis. With this possibility in view, the effect of constipation and catharsis upon urobilinuria was studied in all stages of lobar pneumonia. The results in the different stages differed only in degree, the urobilinuria being modified most by constipation and catharsis when output was greatest.

From several of the cases presented, it has appeared that the presence or absence of fever had a decided effect on urobilinuria. This question was investigated separately and will be reported upon subsequently.

The specific gravity of the urine was taken in every case to determine whether the decrease in urobilinuria following catharsis was due to a lack of

excretion by the kidneys. If catharsis were drastic enough to decrease the amount of urine excreted, the specific gravity should be increased. In all cases it was found that any definite decrease in the amount of urine excreted following catharsis was accompanied by an increase of specific gravity and a reduction of urobilinuria.

In all cases red blood counts, hemoglobin estimations and hematocrite readings were made to determine the possibility of blood destruction as the origin of urobilinuria. These data are not discussed here because the present study concerns itself only with the excretion of urobilin.

These observations show that constipation exaggerates pathologic urobilinuria. The resumption of daily bowel movements reduces this exaggerated urobilinuria to its former level. Catharsis even of moderate degree causes a very pronounced decrease of urobilinuria. The amount of increase attending constipation depends to a large extent on the amount present before constipation began. A high-grade urobilinuria with daily bowel movements is increased much more by constipation than a low-grade urobilinuria under similar conditions. The converse is true of catharsis, for while low-grade urobilinuria is decreased only slightly by catharsis, a high-grade urobilinuria is greatly reduced. Although, owing to other factors, it is difficult to make an exact statement of how much the excretion of urobilin in a given case is affected by constipation and catharsis, a rough estimate may be given. If a patient excretes 15,000 units of urobilin in the urine daily with daily bowel movements, three days of constipation may be expected to increase the daily excretion to 30,000 or 45,000 units. Catharsis at this point sufficient to produce three good bowel movements could be expected to reduce the daily output to between 3,000 and 10,000 units.

These facts suggest that one factor which determines the degree of pathologic urobilinuria is the length of time that the intestinal contents remain in the intestinal tract. When large quantities of urobilin are excreted in the urine, a considerable portion is probably collected from the contents of the colon. It is a striking fact that the degree to which urobilinuria is affected by constipation and catharsis is largely determined by the degree of urobilinuria with daily bowel movements prior to the constipation or catharsis.

CONCLUSIONS

Constipation exaggerates a pathologic urobilinuria. Evacuation of the bowel diminishes it.

REFERENCES

1Whipple: Arch. Int. Med., July, 1922.
2Gerhardt, D.: Ztschr. f. klin. Med., 1897, xxxii, 303.
3Hildebrandt: Ztschr. f. klin. Med., 1906, lix, 391.
4Wilbur and Addis: Urobilin: Its Clinical Significance, Arch. Int. Med., xiii, 235.
5Addis, T.: A Working Hypothesis of Haemoglobin Pigment Metabolism, Arch. Int. Med., 1915, xvi, 413.
6Strouse, H., and Hahn, L.: Zentralbl. f. inn. Med., 1920, No. 11, p. 193.
7Blankenhorn, M. A.: Arch. Int. Med., Feb., 1918, xxi, 282-291.
8Hildebrandt: Die Bedentung der Urobilinurie für Diagnose und Prognose der kruptösen Pneumonie, Ztschr. f. klin. Med., 1911, lxxiii, 189.

LABORATORY METHODS

STUDIES ON LOCAL ANESTHESIA. I

THE USE OF TÜRCK'S REFLEX METHOD TO DETERMINE THE EFFICIENCY FACTOR OF LOCAL ANESTHETICS*

By W. J. R. HEINEKAMP, M.S., M.D., CHICAGO, ILL.

SINCE the skin of the frog is somewhat similar to mucous membrane, it has been used to test the efficiency of local anesthetics. The method, as used, is based on Türck's method of recording reflex time and consists in placing the frog's leg about midway to the knee in a bath of the anesthetic and determining the influence of the drug on the reflex time at definite intervals by stimulating the foot with dilute hydrochloric acid. The frog is decapitated to simplify the reflex. In this series, however, the heart was neither removed as by Sollman[1] nor tied as by Zorn.[2] The fact that the lymph hearts still beat after these procedures presumes that the absorption of the drug is going on and makes this operation unnecessary. Clinically, there is absorption when local anesthetics are applied to mucous membrane, and since this method is an attempt to simulate clinical conditions, removal of the circulation is of questionable value and not indicated. If some absorption occurs, it has no practical influence on the method, since the local action is predominant.

The stimulus used was hydrochloric acid. As shown in Chart 1, the reflex time is dependent on the strength of the stimulus until a point is reached when, regardless of the concentration of the acid, the reaction time remains constant. Any strength above N/5 HCl causes a reflex in one second, that evidently being the minimum reflex time. The strength of the acid is of great importance since a strong solution, e. g., N/1 HCl, such as was used by Issekutz,[3] is corrosive and destroys the sensory nerve endings, or at least alters their activity. An acid in such a concentration is in itself an anestheticum dolorosum. This probably accounts for the results Issekutz obtained.

On the other hand, a minimal stimulus is objectionable in that the reaction time becomes more variable the weaker the acid used (see Chart 2). Hence, to be most reliable the acid should be strong enough to give constant results and weak enough to be noncorrosive. A weaker acid has a decreased osmotic pressure and, hence, would allow a greater flow of the drug from the tissue into the bath. Tenth-normal HCl seems to be best suited for this purpose since the responses are somewhat constant and the acid is not corrosive in this strength for the time it is used.

The length of time the anesthetic acts is of importance since frequent stimulations require considerable washing, which permits and aids the recuperation

of the partially anesthetized skin. A one per cent cocaine hydrochloride solution will paralyze the sensory nerve endings as determined by this method, if allowed to act ten consecutive minutes. However, if the reflex is tested at the end of five minutes and again at ten minutes the paralysis is not complete although the reflex is slowed. We have, therefore, taken ten minutes as the time between tests, realizing that in some cases the result may vary almost this length of time as will be discussed later.

EXPERIMENTS

Designating ten minutes as the time necessary for one per cent cocaine hydrochloride to totally inhibit a reflex with N/10 HCl used as a stimulus (see summary). I found that the drugs used in one per cent solutions have the following comparative values, as shown in the subjoined protocols and tables.

The following protocols exemplify the experiments.

PROTOCOL I
COCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1	Right Leg	10 min.
	Left Leg	10 min.
2	Right Leg	10 min.
	Left Leg	10 min.
3	Right Leg	10 min.
	Left Leg	19 min.
	Average	10 min.

PROTOCOL II
HOLOCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1	Right Leg	10 min.
	Left Leg	10 min.
2	Right Leg	10 min.
	Left Leg	10 min.
3	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL III
ALYPIN 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1	Right Leg	10 min.
	Left Leg	10 min.
2	Right Leg	19 min.
	Left Leg	10 min.
3	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL IV
BUTYN 1 PER CENT

		TIME OF ONSET OF ANESTHESIA
FROG		
1	Right Leg	10 min.
	Left Leg	10 min.
2	Right Leg	10 min.
	Left Leg	10 min.
3	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL V
SALIGENIN 1 PER CENT

		TIME OF ONSET OF ANESTHESIA
FROG		
1	Right Leg	10 min.
	Left Leg	10 min.
2	Right Leg	10 min.
	Left Leg	10 min.
3	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL VI
STOVAINE 1 PER CENT

		TIME OF ONSET OF ANESTHESIA
FROG		
1	Right Leg	20 min.
	Left Leg	20 min.
2	Right Leg	20 min.
	Left Leg	20 min.
3	Right Leg	20 min.
	Left Leg	20 min.
	Average	20 min.

PROTOCOL VII
TROPACOCAINE 1 PER CENT

		TIME OF ONSET OF ANESTHESIA
FROG		
1	Right Leg	20 min.
	Left Leg	20 min.
2	Right Leg	20 min.
	Left Leg	20 min.
3	Right Leg	20 min.
	Left Leg	20 min.
	Average	20 min.

PROTOCOL VIII

BETA-EUCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	20 min.
	Left Leg	20 min.
2		
	Right Leg	20 min.
	Left Leg	20 min.
3		
	Right Leg	20 min.
	Left Leg	20 min.
	Average	20 min.

PROTOCOL IX

APOTHESINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	60 min.
	Left Leg	60 min.
2		
	Right Leg	60 min.
	Left Leg	60 min.
3		
	Right Leg	60 min.
	Left Leg	none
	Average	60 min.

PROTOCOL X

NOVOCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	No Anesthesia in one hour
	Left Leg	No Anesthesia in one hour
2		
	Right Leg	No Anesthesia in one hour
	Left Leg	No Anesthesia in one hour
3		
	Right Leg	No Anesthesia in one hour
	Left Leg	No Anesthesia in one hour

SUMMARY

DRUG	EFFICIENCY FACTOR
Cocaine hydrochloride	1
Holocaine	1
Alypin	1
Butyn	1
Saligenin	1
Stovaine	0.5
Tropacocaine	0.5
Beta-Eucaine	0.5
Apothesine	0.1666
Novocaine	0.0000

DISCUSSION

While this method of determining the relative anesthetic power of various solutions is used, the results are so variant that not much reliability can be placed upon them.

It must be borne in mind that this method measures only the activity of local anesthetics on mucous membrane. Novocaine, which is probably the safest and one of the most efficient local anesthetics in injection work, is practically without action when applied to mucous membrane. (Hence, the only conclusion that can be drawn from the results obtained by the use of this method is that the drug under consideration is or is not a good anesthetic for mucous membrane. Other factors such as toxicity, corrosiveness, etc., are disregarded.)

VALUES.
N/1 = 1.
N/5 = 1.
N/10 = 0.3667
N/20 = 0.2467
N/40 = 0.1170
N/50 = 0.1087
N/100 = 0.0632
N/150 = 0.0211
N/200 = 0.0000

THEORETICAL THRESHOLD
NORMAL/180 HYDROCHLORIC ACID
AVERAGE of 185 EXPERIMENTS
1 SECOND = MINIMUM REACTION TIME.

CHART I
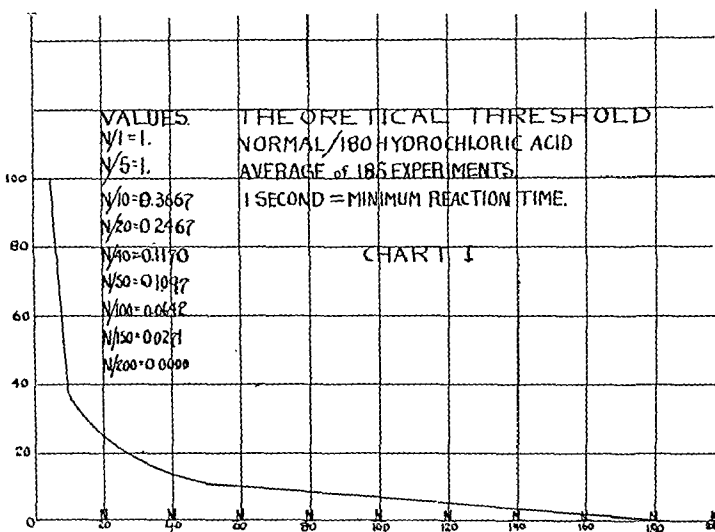
The duration of anesthesia cannot be measured by this procedure. While the nervous system of the frog apparently recovers quickly from the shock produced by decapitation, we must consider that the cord and the medulla suffer some molecular disintegration which predisposes to their inactivity. This is noticed even more when the circulation is tied off, since the nervous elements then react quickly to asphyxia and starvation. It cannot be concluded that the frog is still under the influence of the anesthetic when a reflex is not obtained. The nervous system might be dead. Hence, this method does not furnish any data concerning the duration of the anesthetic.

In a certain number of frogs, regardless of the amount of brain destroyed, swimming movements that simulate the normal reflex are present. It is pos-

sible for the skin to be completely anesthetized and still have swimming movements result on immersion of the foot into the acid. Hence, a false reflex may alter the efficiency factor as determined by this method.

The time is not accurate, in that nine minutes difference may actually be present in the anesthetizing time of two drugs while the efficiency factor would be the same. For example, drug A does not paralyze in ten minutes but does in twenty. There is no way of determining whether paralysis was effected in eleven or nineteen minutes.
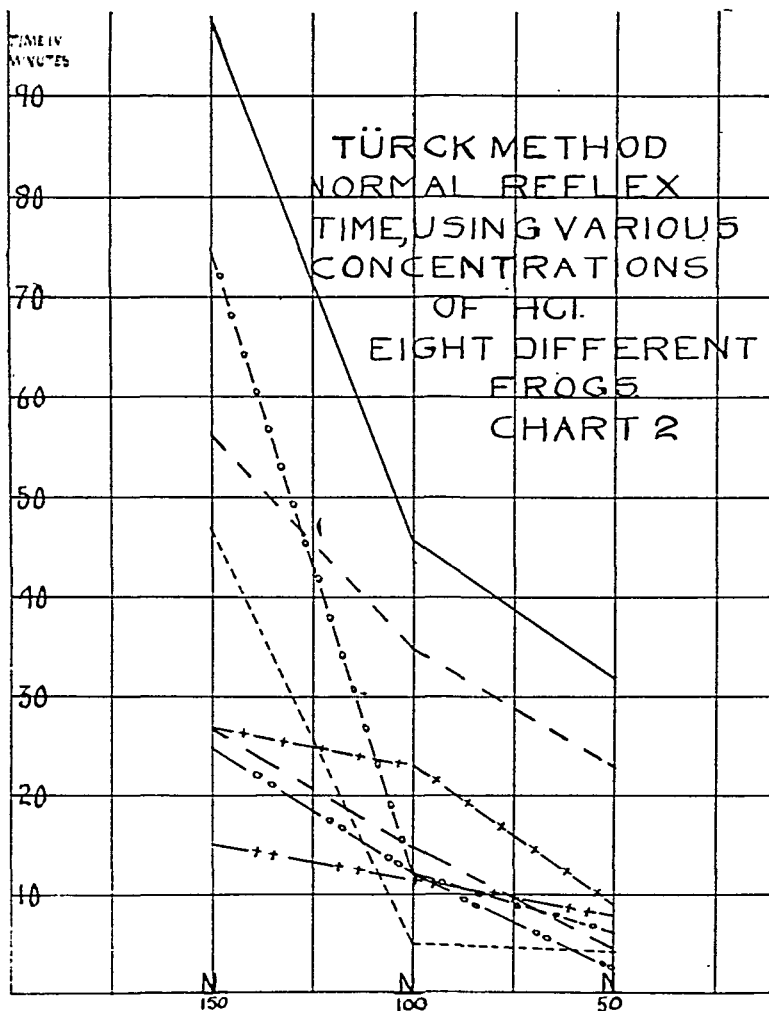
We found that ten minute intervals were optimum for the determination of the reflex time. Cocaine, which we used as a standard, did not paralyze in ten minutes if the reflex was taken at five minutes and again at ten minutes. It is necessary for cocaine to act ten consecutive minutes. Apparently the washing removes enough of the drug so that the action is deferred. Hence, a ten minute interval is necessary. It is not improbable that a drug failing to act in ten minutes but producing paralysis in twenty minutes, would effect complete anesthesia if allowed to act fifteen consecutive minutes.

Since frogs react differently in various seasons we feel that the results depend in part on the time of year the experiments were completed.

Chart 2 shows the results obtained from eight experiments done under the same conditions. The wide variations in reactions increase as the strength of the acid becomes weaker. Hence, we feel that any conclusions must be based on the average of a large number of experiments. Chart 1 is a curve based on a series of 185 experiments. We find that while N/180 HCl is not the absolute threshold, very few frogs react with it as a stimulus.

CONCLUSIONS

While this method is simple and easily performed the disparity in results shows that reliable data cannot be obtained. The method is of little value in the comparative work and of questionable value from a practical standpoint.

REFERENCES

[1]Sollman: Jour. Pharmacol. and Exper. Therap., 1918, xi, 1.
[2]Zorn: Ztschr. f. exper. Path. u. Therap., 1923, xii, 529.
[3]Issekutz: Arch. f. d. ges. Physiol., 1912, cxlv, 448.

A METHOD FOR SAMPLING AND ANALYSIS OF STOMACH GAS*

By A. D. Dunn, M.D., and F. L. Dunn, M.D., Omaha, Nebraska

IN a study of the carbon dioxide and oxygen content of stomach gas in normal persons,[1] we endeavored to learn the volume per cent ratios of these two gases in gas aspirated from the stomach of healthy individuals under varying conditions. It was shown that atmospheric air introduced into the stomach tends to approach equilibrium with the blood gases within one hour in the case of carbon dioxide, and considerably later in the case of oxygen. In this work we repeatedly met with unaccountable sudden variations in the volume per cent of carbon dioxide, and oxygen in successive samples from the fasting stomach obtained at intervals of five to twenty minutes. The explanation for these sudden variations would seem to lie either in (1) swallowed air, or (2) in sudden regurgitating of duodenal gas, or (3) in indeterminable factors affecting the rates of liberation of carbon dioxide, from and the absorption of oxygen into the stomach wall, or (4) in possible partitioning of the gas bubble, or (5) in two or more of these factors combined. From roentgenologic investigations, appreciable duodenal regurgitation of gas would seem to be negligible. In any event, whatever gas might be passed from the duodenum into the stomach should in normal individuals at least approximate the composition of stomach gas. The one factor susceptible of experimental control is the swallowing of air; a study of this factor involves a brief review of methods heretofore used in the investigation of stomach gas.

Planer (1860)[2] was the first to make any accurate attempt at the analysis

of stomach gases. His work was confined to the examination of gas samples obtained from the stomachs and intestines of dogs recently killed.

Schierbeck[3] repeated Planer's experiments and studied the gases found in the stomach and intestines of human bodies immediately after death. He tried to estimate the carbon dioxide in the living by bubbling the gas from stomach contents aspirated with water through a standard barium hydroxide solution. His last method has to do only with gas which can be driven off from the stomach contents and does not apply to gas contained in the stomach apart from food.

Hoppe-Seyler[4] collected gas by connecting a three-stoppered inverted bottle filled with water with a stomach-tube. The gas was collected over water and transferred to a Hempel burette for analysis. Later he collected gas directly into a tube, transferring it to a Hempel burette and analyzing it by Hempel's method. This work was done almost entirely on pathologic material and his normal gas values indicate that air swallowing was not controlled.

Woodyatt and Graham[5] first introduced room air into the normal stomach, withdrawing it in five minutes for analysis. This method was used merely to establish the liberation of carbon dioxide into the stomach by the gastric mucosa.

Yllpö[6] studied the changes taking place in air, carbon dioxide and oxygen introduced into the fasting stomach. A 1 cm. stomach tube was used both to introduce and to sample gas. The difficulty of *voluntarily* controlling air swallowing was mentioned but no *mechanical* means was employed to overcome this error. His average normal value, 4.4 per cent carbon dioxide, indicates that air swallowing played a not unimportant rôle.

Kantor[7] used the rather crude Hoppe-Seyler method in a "few preliminary estimations undertaken on an unselected series of individuals aspirated at various intervals after eating" in which no mention was made of the unknown factor of air swallowing. His low carbon dioxide values suggest that equilibrium of this gas with air swallowed with the sampling tube, had not been reached before samples were removed for analysis.

It was obvious from a cursory review of the literature that reliable quantitative methods for the study of stomach gas obtained from living human subjects had not yet been developed. No attempt had been made to determine the rate of gaseous interchange within the stomach. Little effort had been made to learn the effect of swallowed air on stomach gas concentrations. In upwards of three hundred gas analyses made on fifty different individuals, normal and abnormal, we have developed a method which seems to meet the requirements of sound experimental procedure for obtaining and analyzing stomach gas. The important features of this method are the introduction of a measured volume of air of known composition into the stomach, the restriction of sampling to the fasting stomach in studying normal gaseous interchange, the prevention of air swallowing, and sampling at frequent intervals.

The introduction of a measured quantity of air of known composition into the stomach in order to facilitate sampling has obvious advantages. Difficulty in sampling from insufficient air bubbles is common. The average size of the air bubble is about 50 to 100 c.c. Its position is often out of range of the sam-

pling bucket. One hundred fifty to two hundred c.c. of air introduced into the stomach insures several adequate samples. The taking of a deep breath by the descent of the diaphragm will often make a gas bubble accessible to the sampling bucket. The introduction of air into the stomach is also of value in determining
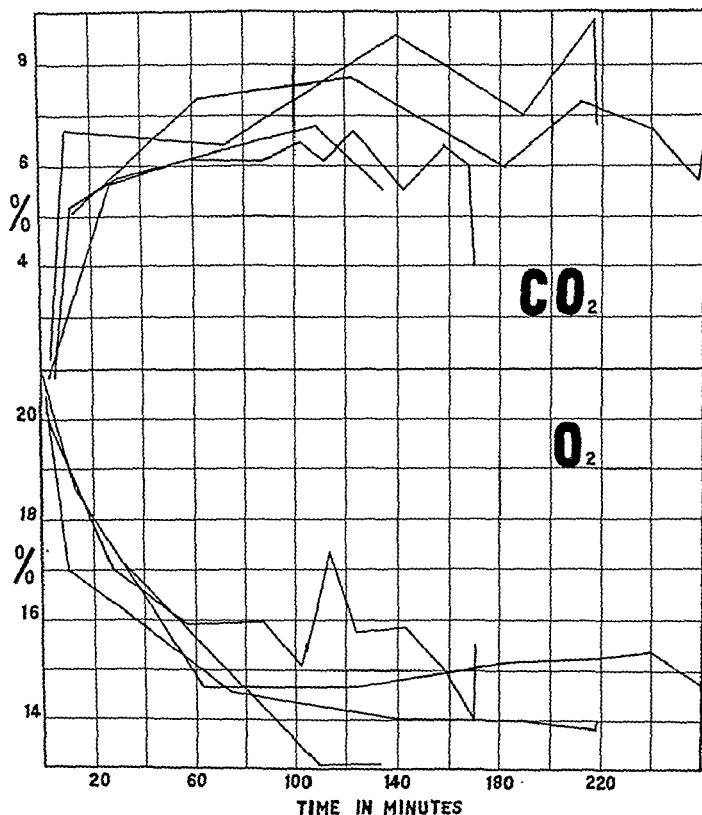
Fig. 1.—Four curves showing sudden variations frequently encountered when only voluntary efforts are made to control the swallowing of air. It is to be noted that the greater variations occur in the carbon dioxid curves

the rate of liberation of carbon dioxide into, and of absorption of oxygen from, the stomach.

Sampling is done largely on the fasting stomach. The admission of food soils the apparatus, tends to disturb timed sampling and introduces unnecessary variables such as the possible effects of postprandial variations in alveolar air

as described by Dodd.[8] Inasmuch as it would seem at first desirable to determine the rate of liberation of CO_2 into the stomach, the advantages for this purpose of the fasting stomach are clear. However, a series of analyses made from digesting stomachs of the same subjects, failed to reveal noteworthy differ-
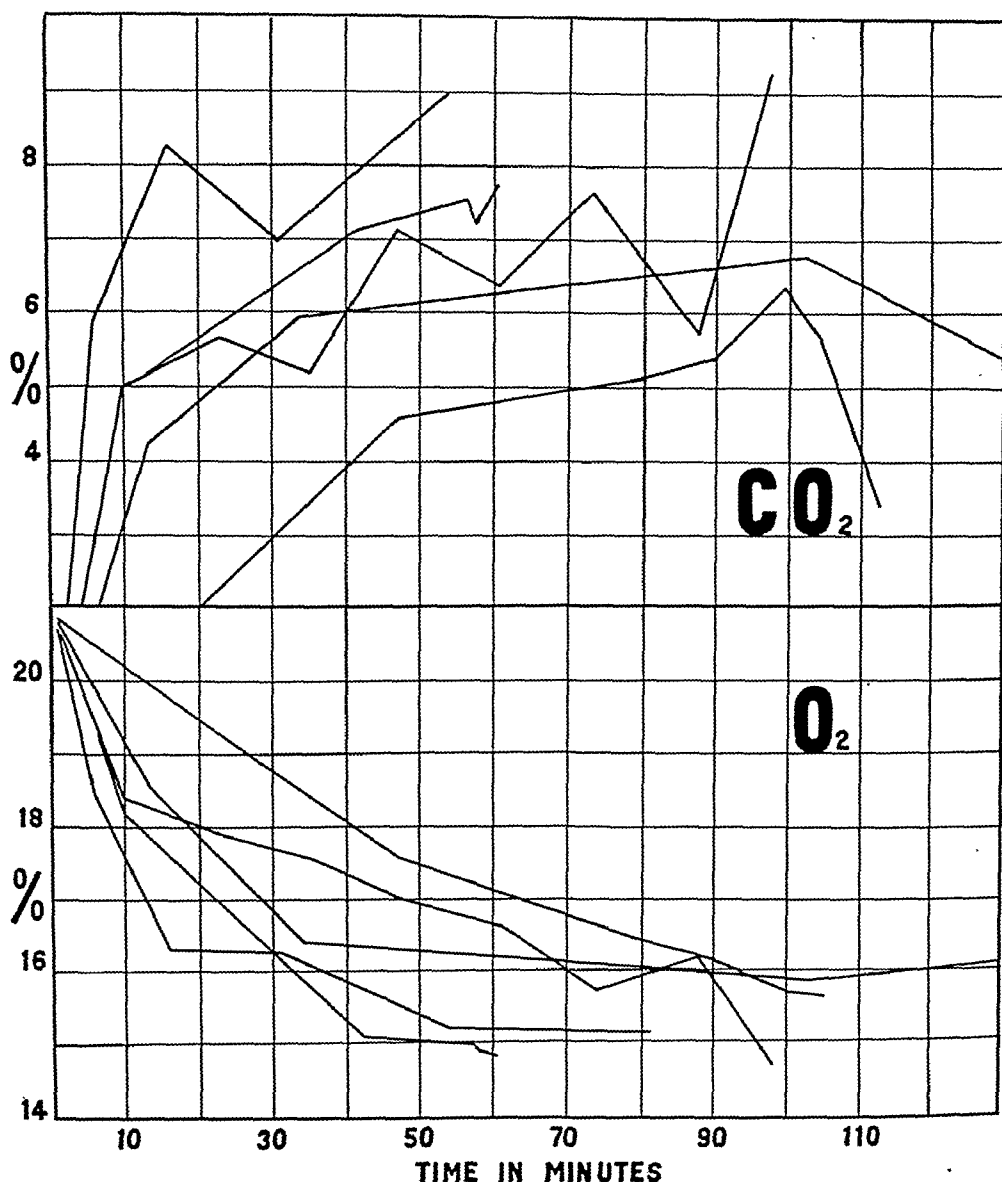
Fig. 2.—The lack of smoothness in the curves is similar to that noted in Fig. 1. An effort was made to prevent ingress of air into the stomach by constant pumping of saliva and air out of the esophagus.

ences in the percentages of carbon dioxide and oxygen. Fermentation is a minor if not a negligible factor in the production of stomach gas, except possibly in the absence of free hydrochloric acid. In the study of pathologic conditions, it will undoubtedly be necessary to determine with accuracy what effect the presence of food in the stomach may have on gas production. For the sake of sim-

plicity, however, it seemed wise for the present to confine our work to the fasting stomach.

Swallowing of air must be controlled. Only in exceptional instances have we been able to obtain curves of expected smoothness without mechanical prevention of air swallowing. Fig. 1 shows the irregular curves usually obtained when a single Rehfuss tube was used in sampling and only voluntary efforts made to control air swallowing. There is an unconscious passage of air and saliva into the esophagus which cannot be satisfactorily controlled by voluntary effort. We first attempted to get rid of swallowed air by cementing a per-
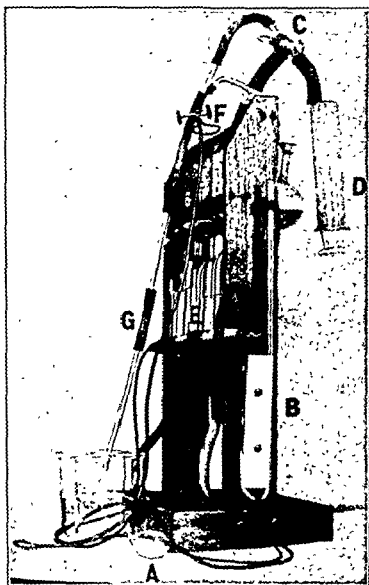
Fig. 3 —*Sampling apparatus with balloon to prevent air swallowing, description to be found in the text*

forated duodenal tube to the aspirating tube. This second tube was fixed to the aspiraing tube so that its lower end, which was freely perforated, would rest in the lower esophageal ampulla. It was attached to a water suction pump and negative pressures of 40 to 100 mm. of mercury were used. Fig. 2 shows that the results obtained by this method were no better than by the single tube method. It still seemed that air might enter the stomach in spite of suction as air incorporated in small bubbles in saliva. A distensible rubber balloon placed just above the cardia seemed the most likely solution of the swallowed air problem. X-ray studies indicated that the balloons should have a distensibility of 3 to 5 cm. A balloon pressure of 25 to 50 mm. of mercury depending on the

elasticity of the balloon was needed. It is likely that the balloon acts somewhat after the manner of a water lock, at times permitting accumulated saliva to pass, carrying minute bubbles of air, to be sure, but apparently not in sufficient
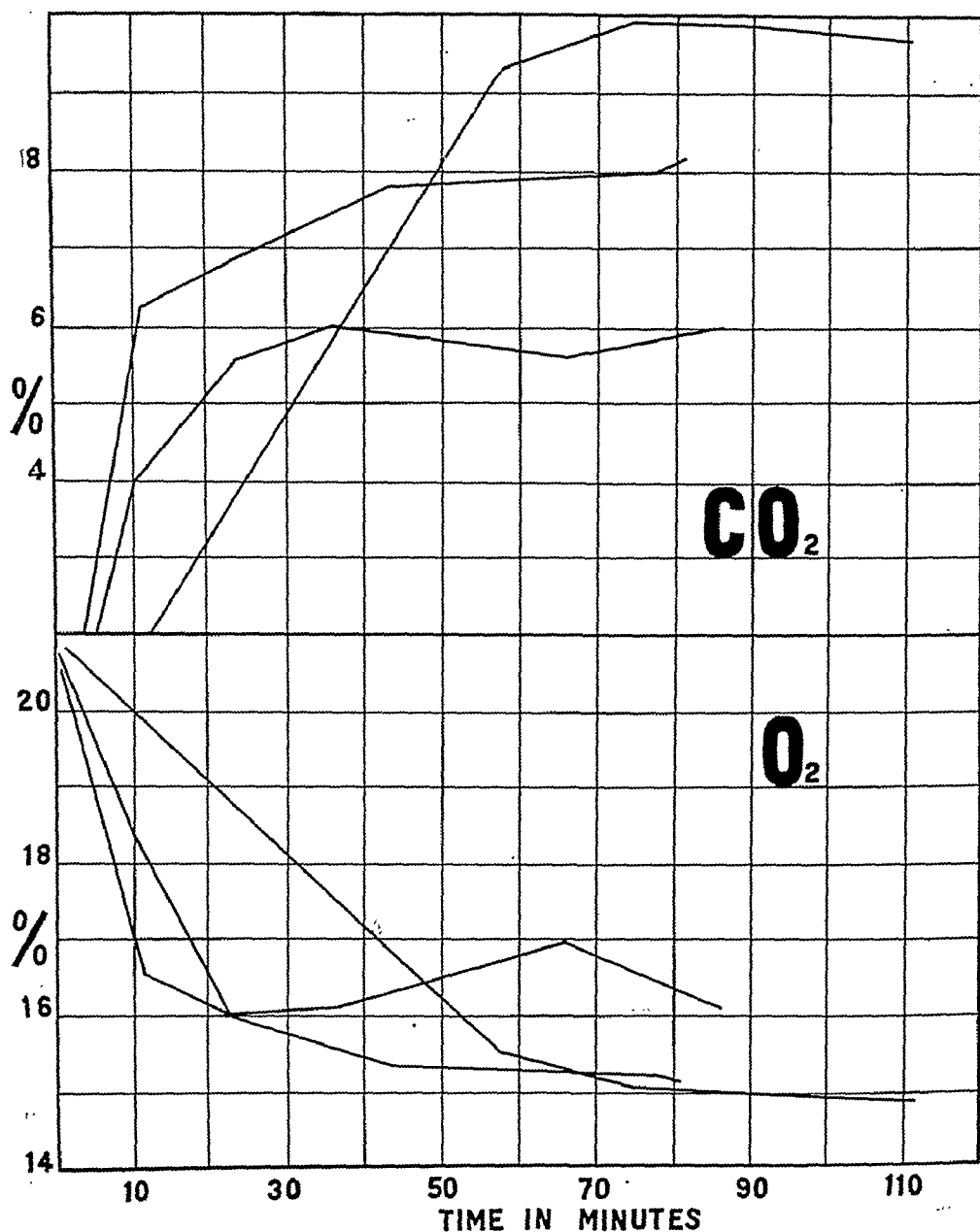
Fig. 4.—Typical illustrations of the curves obtained when air swallowing is prevented by the distensible esophageal balloon.

amount to vitiate results. The discomfort to the subject is little if any more than that experienced in fractional test meals or nonsurgical biliary drainage. Fig. 3 shows apparatus with aspirating bucket and balloon with sampling tube

and manometer attached. Fig. 4 shows characteristic curves obtained by this method.

In brief the method of obtaining gas is as follows: Fig. 3 illustrates the collecting apparatus ready for use. It consists of a Rehfuss tube with a small balloon (*A*) attached about 10 cm. from the bucket, a manometer and test tube (*B*) connected to the balloon, a three-way stopcock (*C*), Luer syringe, (*D*) and sampling tubes (*E*) for collecting gas samples. The balloon is tested for leaks and for the pressure required for the desired distension. The tube is introduced to 42 to 48 cm. at which level most gas bubbles can be tapped. The balloon is then inflated. The stopcock (*C*) is turned so that Rehfuss tube and syringe are in communication, the clamp (*F*) removed and gentle suction is applied. Fasting contents are then drawn into the syringe for fermentation tests, the stopcock (*C*) is turned so that syringe and waste pipe (*G*) are in communication and the fasting contents forced into a test tube. The syringe and Rehfuss tube are then put in communication and fasting contents removed until the gas bubble is tapped. Then the stopcock (*C*) is turned so syringe (*D*) and waste pipe (*G*) are shut off, the stopcock on the sampling tube opened, and the gas sample collected by lowering the mercury reservoir. If insufficient gas is obtained or if so desired 150-200 c.c. of gas of known composition is introduced via *G C D C A*. After the collection of each sample, acidulated water (½ per cent sulphuric acid) is run into the Rehfuss tube and its connections until all the air has been displaced. As shown in Fig. 3 each of the sampling tubes (*E*) also contain 10-15 c.c. acidulated water. By this means the samples are collected uncontaminated. In transferring the collecting apparatus from one sampling tube to the next the clamp (*F*) is closed. Samples are collected at five to thirty minute intervals as desired.

A Pettersen-Palmquist apparatus was first used for gas analysis, but on account of the large amount of gas necessary (25 c.c. for each analysis) and on account of the desirability of analyzing successive samples obtained at brief intervals of time this machine was discarded in favor of a Boothby modification of the Haldane Gas Analysis machine for carbon dioxide and oxygen. Although the latter machine is accurate to a few hundredths of one per cent, no attempt has been made to get results better than correct to one-tenth of one per cent. When a sufficient sized sample was collected the mixing method described by Boothby and Saniford[9] was used. When the sample was too small Haldane's[10] original technic of displacing all the air in the system by mercury was employed.

<center>COMMENT</center>

It is apparent that the methods heretofore used in the study of stomach gases are open to criticism. The study of gas as obtained postmortem or from freshly killed or anesthetized animals cannot be expected to give the same representative values as when obtained from subjects sitting quietly in a chair or lying in bed. Furthermore, no effort has been made to determine whether the samples collected from living human subjects were representative of the equilibria for these subjects or whether such samples consisted appreciably of air swallowed in the sampling process. Given an accurate method of obtaining and

analyzing stomach gas, much work must be done before a conception of the physiologic pathology of the symptom of "gas on the stomach" can be formulated. *The method opens up many problems for investigation.* From our limited observations on supposedly normal subjects with the above method, it would seem that in atmospheric air introduced into the fasting stomach, the carbon dioxide rises rapidly and that in twenty to thirty minutes the curves flatten out and hold somewhere between 6 and 9 volumes per cent. In other words there is a stomach respiration. Many observations must be made in order to establish the limits of the normal curves if such misconceptions as once prevailed in regard to hydrochloric acid are to be forestalled. The behavior of stomach gases in disease is relatively unknown. While we have made numerous studies of patients complaining of stomach gas and obtained results of interest, yet sufficient observations have not been made to venture any conclusions. The reaction of the stomach to the introduction of various gases such as carbon dioxide, oxygen, nitrogen, etc., should also be of interest.

CONCLUSIONS

1. A more accurate method has been described for the collection and analysis of stomach gas with reference to its carbon dioxide and oxygen content, permitting of a study of the equilibrium relationships existing in normal subjects.

2. Analyses made to date show that in air of known composition introduced into the stomach of supposedly normal subjects the carbon dioxide and oxygen contents tend to come into equilibrium at relatively constant values.

REFERENCES

[1]Dunn, A. D., and Thompson, Warren: Arch. Int. Med., Jan., 1923, xxxi, 1-8.
[2]Planer: Sitzungsberichte d. Mat. Natur. Cl. d. k. Akad. d. Wissensch., Wien., 1860, quoted by Schierbeck.
[3]Schierbeck, M. P.: Ueber Kohlensäure im Ventrikel, Skand. Arch. f. Physiol., 1892, iii, 437.
[4]Hoppe, Seyler G.: Deutsch. Arch. f. Klin. Med., 1, 82.
[5]Woodyatt, R. T., and Graham, E. A.: Alimentary Respiration, Tr. Chicago Path. Soc., 1912, viii, 353.
[6]Yllpö, A.: Ueber Magenatmung beim Menschen, Biochem. Ztschr., lxxvii, 273.
[7]Kantor, J. L.: A Study of the Atmospheric Air in the Upper Digestive Tract, Am. Jour. Med. Sc., 1918, clv, 829. Kantor's excellent article contains a critical review of the literature and theories on stomach gas; it can be recommended for a quick survey of the subject.
[8]Dodd, E. C.: Jour. Physiol., 1921, liv, 342.
[9]Boothby, W. M., and Saniford, Irene: Basal Metabolic Rate Determination, p. 70, W. B. Saunders Co., Philadelphia, 1920.
[10]Haldane, J. S.: Methods of Air Analysis, p. 18, Chas. Griffin & Co., London, 1918.

A MODIFICATION OF THE MOIST CHAMBER*

By Alfred Reich, Cleveland, Ohio

A SIMPLE and economical device for the study of tissue, fungi, and micro-organisms in general, *in vitro*, over a prolonged period of time, and under high magnification, can be made by grinding holes five-eighths of an inch in diameter, through the bottom of a Petri dish, and sealing large cover slips over the openings at the outer surface by means of balsam or some other adhesive material (Fig. 1). The dish is then covered and placed, inverted, in a large Petri dish, which is in turn covered with adhesive tape and placed in an auto-clave. On removal from the sterilizer, the device, which throughout these operations is kept in a horizontal position with the inner Petri dish inverted, should be placed in a cool atmosphere to permit the balsam to congeal.

X-Section of Plates

View looking down on inner Petri dish
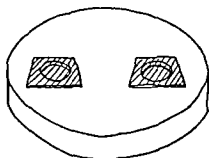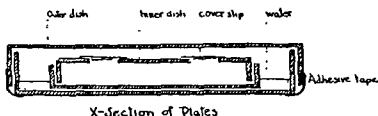
Fig. 1.

To use this device, the inner dish is removed from the larger one, and placed upright on a flat surface. By lifting the cover of the dish as one does to streak a plate, a thin film of the desired liquid medium may be spread into one of the ground-out recesses of the dish by means of a sterile platinum loop or capillary pipette. The medium may then be inoculated with the desired organisms, after which the smaller Petri dish is replaced in its original inverted position in the larger one. A small quantity of water is then placed in the larger dish to prevent the evaporation of the medium, the cover is again secured with adhesive tape, and the whole incubated at a suitable temperature. The effect is the same as that secured by the well-known moist chamber, except that a constant supply of air is assured when needed, manipulation is much simpler,

and the chances of contamination are appreciably lessened. The thinness of the cover-glass permits observation through the highest power.

If for any reason a solid medium is desired, as for the study of bacterial colonies, we have found it advisable to seal the cover slip to the *inner* surface of the dish, proceeding with the sterilization as described above. A very thin layer of melted medium, at as low a temperature as possible, is poured into the plate, and the inoculation is made over the site of the cover slip; or the tube of melted medium may be inoculated and then poured into the plate, with a strong probability that some of the colonies will occur at the site of the cover slips. Incubation is done as described above, observations being made at daily or more frequent intervals.

The Gorgas Memorial Institute

TWENTY-FIVE million cases of illness every year in the United States are a challenge to the whole medical profession, especially when it is considered that at least 20 per cent and perhaps 40 per cent of such illness is believed preventable.

The profession is aware of the havoc wrought by infections of the oral cavity, and appreciates that eradication of such infections means less illness. The cooperation of dentists with the Gorgas Memorial Institute is significant of an era in which the fight to conquer disease will be backed by a triple alliance—physician—dentist—individual.

The 35,000 dentists who are members of the American Dental Association have joined with the Gorgas Memorial Institute in its work of decreasing preventable illness and the consequent premature deaths which result from ignorance, carelessness, and lack of hygiene.

While the support of the American Dental Association is encouraging to the professions involved, such action also bodes well for the millions who harbor infections of the oral cavity. Many such persons need the services of the physician after their condition has been detected by the dentist. And by the same token, physicians will take precautions to see that their patients get the needed dental care which their condition demands.

EDITORIALS

Dengue Fever

DENGUE fever which is endemic in our southern states not infrequently reaches the proportions of a terrific epidemic; its incidence and rapidity of extension exceeding even that of influenza. Chandler and Rice (1922) estimated that the epidemic of 1922, in Texas, claimed between 500,000 and 600,000 victims. Fortunately dengue is never fatal, but it is disabling and during the disease the patient suffers excruciatingly. As a cause of disability alone, even though we should consider the average days lost as only five, the cost of this one epidemic to the State of Texas was enormous. Recently there has been an epidemic in India, possibly five or more times the size of our own. In the Philippines, dengue affects chiefly the newcomers—natives seem to have a high degree of acquired immunity. The visitors affected are largely Army and Navy people, the families of those sent there for duty. Such persons sometimes come down with dengue within one week of their arrival in Manila.

Dengue is, therefore, a disease of no mean importance from a military standpoint. Its possible interference with emergency maneuvers could be most serious in any campaign involving the transfer of troops from regions where dengue does not usually exist, into an endemic center. It is not surprising, therefore, that among the problems taken up by the U. S. Army Medical Department Research Board in Manila, dengue has had an important place and the report of the work (1925) done upon it shows that several of the important points in the epidemiology and mechanism of transmission of dengue have been cleared up for all time, and our knowledge of the disease has been greatly enlarged.

The conditions under which the work was done made it possible to control those factors which have cast doubt upon previous dengue work along the same lines. The results, therefore, are clear cut and enough of the experimental details are given in the preliminary report to show that the work has fundamental value.

As experimental material, forty-two American soldiers volunteered for the transmission experiments and dengue was caused experimentally by the bites of mosquitoes in twenty-five instances.

It will be remembered that Graham, in 1903, first reported the transmission of dengue by *Culex* mosquitoes. It is evident that not all the mosquitoes in his experimental lot were *Culex* however, and his experiment was not well controlled.

It will also be recalled that Ashburn and Craig (1907) first showed that dengue can be transmitted by injecting the blood of a patient into a susceptible individual and that the cause of dengue is filtrable; they produced the disease through the injection of filtrates of the blood. In their mosquito transmission work, one of their cases indicated that *Culex* as well as *Aëdes* (*Stegomyia*) could transmit dengue but they have themselves wanted this confirmed, the conditions of the experiment not having been satisfactory to them. Cleland, Bradley and MacDonald (1918) demonstrated quite definitely that *Aëdes egypti* is a transmitter of dengue. The question as to the potential rôle of *Culex* therefore needed to be cleared up. The knowledge that *Aëdes* is able to transmit dengue needed to be amplified and further information concerning the exact mechanism of transmission was required before our information could be applied in prevention work.

All the mosquitoes used by the Board were bred from the eggs and in each experiment the exact number used was known; exact counts of those that actually took blood were made following each contact with a volunteer. The volunteer subjects of the experiment were kept in strict isolation in a special ward of the Sternberg General Hospital. The ward was entered through a series of doors in a screened vestibule and all screens were examined every day to be certain they were in perfect condition. The volunteers were held under observation for a sufficient period, before subjecting them to the bites of the mosquitoes, to be certain they were not already infected.

Dengue fever was transmitted by the bites of infected *Aëdes egypti* mosquitoes twenty-five times. The *Culex* failed to produce dengue in five patients under the same conditions and after waiting for a sufficient time to

cover any possible incubation period, the same persons were bitten by *Aëdes*—all then came down with dengue. *Culex quinquefasciatus,* therefore, is finally ruled out as a potential transmitter of dengue fever.

The dengue patient is able to infect mosquitoes during the twenty-four hours preceding the onset of symptoms and during the first three days of the disease, but not later. The virus seems to be present in the circulating blood in highest concentration on the first day of the disease.

Just as in yellow fever, the virus must remain in the mosquito eleven days before the bite becomes infective, but this period past, the *Aëdes* remains infective for a very long time—probably throughout the rest of its life.

Immunity studies indicated that one attack confers a certain degree of resistance but this gradually disappears. Second and even third attacks in the same individual are not at all uncommon and the relatively high immunity of natives can be accounted for best by the assumption that they are frequently reinfected.

With this exact information now at hand, it is possible to direct efforts at prevention along definite lines. The *Aëdes egypti* mosquito is a most difficult one to control with methods available at present but with studies directed specifically against it, who can say what the result may be? Patients should be screened from mosquitoes during the first three days of the disease. If this is done the incidence of dengue will certainly be reduced. The factor in epidemiology most difficult to control—impossible, in fact—is the *infection of mosquitoes during the last twenty-four hours of the incubation period.*

REFERENCES

Ashburn and Craig: Experimental Investigations Regarding the Etiology of Dengue Fever, Philippine Jour. Sc., 1907, ii, 93-152.
Cleland, Bradley, and MacDonald: Dengue Fever in Australia, Jour. Hyg., 1918, xvi, 317-418.
Graham, H.: The Dengue. A Study of its Pathology and Mode of Propagation, Jour. Trop. Med., 1903, vi, 209-214.
Siler, Hall, and Hitchens: Results Obtained in the Transmission of Dengue Fever, Jour. Am. Med. Assn., Apr. 18, 1925, lxxxiv, 1163.

—*A. P. H.*

Erratum

The title of the article by Graham and MacCarty on page 548 of the April issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE was printed incorrectly through an error not the fault of the authors. The words "Spinal Cord" should read "Spinal Fluid"—"On the Application of the Hench-Aldrich Urea Index to the Spinal Fluid."

BOOK REVIEWS

Practical Lectures*

THE volume is a compilation of papers presented before the Medical Society of the County of Kings, Brooklyn, N. Y. In every sense it lives up to its title. The contributors are predominately practicing physicians and surgeons who have obtained preeminence in their individual fields and who in their lectures have endeavored particularly to give points of practical importance and interest to the practicing physician. The titles of the individual papers give a good idea as to the character of their contents. Examples are: "The Surgical Abdomen" by Dr. Joseph A. Blake; "Surgical Diagnosis" by John B. Deaver; "Rational Pathology in the New Therapeutics" by J. W. Ewing; "The Common Skin Diseases" by John A. Fordyce; "Diagnosis of Abdominal Tumors" by Russell S. Fowler; "Diagnosis and Treatment of the More Common Recto-Colonic Diseases" by Samuel G. Gant; "Renal Function in Clinical Medicine" by Herman O. Mosenthal; "Everyday Bacteriology" by Wade W. Oliver; "Office Orthopedics" by Walter Truslow; "Fundamental Signs in the Examination of Patients" by Luther F. Warren. In all there are twenty-five contributions.

We cannot recommend this book too highly to practicing physicians as a reference volume covering recent advances in general diagnosis and treatment.

Goiter: Nonsurgical Types and Treatment†

WE find this book apropos in view of recent developments in the nonsurgical treatment of the various types of goiter. The author takes the stand that a large percentage of goiters surgically treated are perfectly amenable to nonoperative procedure. His primary purpose is to assist the general practitioner in classifying his cases into surgical and nonsurgical types, and to indicate what methods bring about the best and promptest results in those instances obviously of nonsurgical nature. Among the nonsurgical diseases of the thyroid, he mentions simple parenchymatous hypertrophy, colloid goiter, puberty hyperplasia, and the hyperplastic swelling of Graves' disease. Surgical goiter embraces adenoma, cystic goiter and other types of thyroid enlargement, not classified under nonsurgical goiter. In general, with the exception of strumitis, malignant goiter and diffuse adeno-

matosis, surgical goiters are encapsulated while nonsurgical goiters are diffuse or unencapsulated. An unencapsulated swelling of the thyroid gland implies a physiologic necessity for more thyroid hormone elsewhere in the economy or it indicates a defensive reaction against toxins during the existence of a focal or general infection. Encapsulation of a goiter is an indication that physiologic adaptation has ended and a pathologic reaction has begun. Pathologic or surgical goiters begin usually as physiologic or nonsurgical swelling, which, had they been treated at the proper time, would have been cured without surgery.

The major portion of the volume is devoted to exophthalmic goiter. The author summarizes the beneficial effect of iodine and stresses the use of quinine more than has been done recently by other authors. Many drugs are discussed pro and con. The author presents an excellent tabular differentiation between the various types of goiter.

Gonorrhea*

MIDWAY between the textbook, in which the author eliminates his own personality and the monograph in which he devotes practically his entire attention to his own work and theories, pausing to be sure, to compare these with the contributions of others, we find a group of contributions in which the author, while naturally giving major attention to his own work and theories, devotes a large amount of space to considerations of more general interest. The reader here finds himself possessed with an excellent general treatise on a particular subject, one which gives due credit to the various theories and experiments of other workers but which views the subject as it were through the spectacles of the author.

Dr. Thompson with the help of his associates has made a very distinct contribution to the literature of gonorrhea. The 500 pages are allotted as follows: 145 to bacteriology; 98 to anatomy, pathology, cytology, and clinical pathology; 40 to immunity and the complement-fixation test; 88 to vaccine therapy, serum therapy, chemical therapy, electro and thermo therapy; 14 to prophylaxis and abortive treatment, and 105 to clinical manifestations and the practical modern treatment of gonorrhea. This proportionate distribution gives a well balanced whole. Although the volume is a British contribution, the recently developed American methods of treatment receive due consideration.

Angina Pectoris†

WERE it not of tremendous intrinsic value, this work would even so be highly prized as the last contribution of the late Sir James MacKenzie, Dean among "family physicians" of the older school.

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*Gonorrhea. By David Thompson, O B E., M.B., Ch.B., Edin , D.P.H., Camb With contributions by David Lees, D.S.O.; Claude H. Mills, M.R.C.S., Eng.; Robert Thompson, M B., Ch.B., Edin ; Kenneth Maclachlan, M. B., Ch.B., Edin. Illustrated. Pp. 519. Cloth. Price $12.75. Henry Frowde and Hodder and Stoughton, London.
†Angina Pectoris. By Sir James MacKenzie, M D., F R S , F.R C P , LL D , Ab and Es F.R.C.P.I. (Hon). Cloth. Pp. 253. Price $9.00. Henry Frowde and Hodder and Stoughton, London, 1923.

The work is a true monograph in which the author presents a summary of his own original researches and his conclusions drawn therefrom. He makes no attempt to discuss other theories and gives few references to the work of others. At the outset he points out that in reading the literature of angina pectoris, we can but be struck with the number of untrue assertions made. Hypotheses are put forward supported by meager evidence or even without evidence. Such hypotheses are often taken as truths, so much so that methods of treatment are based upon them. Throughout the monograph, Dr. MacKenzie attempts to make no statement without clearly indicating the reasons therefor. His many years devoted to a study of pain are applied to the consideration of pain in angina. He stresses the knowledge that a correct understanding of angina will not be reached until we have attained a correct understanding of heart failure. The mechanism causing pain in angina is present in all individuals and the pain is elicited as a result of disturbed reflexes. It is muscular in origin according to Dr. MacKenzie's conception.

The importance of evaluating prognosis is stressed and prognosis is discussed in great detail.

Probably of foremost interest is the enumeration of the multiplicity of possible causes for the symptom known as angina and the discussion of the pathologic changes associated with these causes.

Internal Secretion and the Ductless Glands*

INTO the midst of a riot of unsupported theory and fancy on the action of the glands of internal secretion, there appears a contribution by a leader in the study of endocrinology, a contribution which should serve as a valuable "brake" in the hyperenthusiasm of others. The book is devoted to a critical study of those experimental facts which have been definitely demonstrated and to a discussion of the conclusions which may logically be drawn therefrom without the advancement of new all-inclusive and all-explaining theories.

Particularly in the interaction between the various ductless glands does the author caution against error in interpretation. "If there has been in many instances undue haste in formulating theories of the functions of individual glands, much more has this been the case in regard to theories of interrelationship. It is not certain that the clinicians have been greater offenders than the laboratory workers."

Major attention is devoted to the work on the internal secretion of the adrenal bodies, the thyroid and parathyroid and the pituitary body, although the internal secretion of the reproductive organs is given considerable space.

Where no conclusive argument has been promulgated, the author contents himself with the recording of observations. This applies for example in a discussion of the hypothetic internal secretions from the kidney and prostate.

*The Physiology of Exercise**

APPEARING as a volume in "The Physical Education Series" edited by R. Tait MacKenzie, the first portion deals with the general effects of exercise upon bodily functions. Exercise is divided into various types. The active type includes the exercises of speed, exercises of endurance, of strength, skill, attention, and alertness. Support type of exercises consists of exercises on the feet and in the head-down position. Passive types include massage and passive movement. Finally, the author discusses postural types of exercise. In the physiologic consideration, chief attention is perforce given to the circulation, the blood pressure and the respiration, although chapters are also devoted to the blood and the neuromuscular mechanism.

In the second part of the book, we find the practical application of the points brought out in the first section with divisions devoted to gymnastics, athletics, aquatics and to physical efficiency tests. The physiology of training as studied by the author in the training of large groups of athletes is discussed in detail.

The book comprises a practical application of the studies of the author as director of physical education at the International Y. M. C. A. College at Springfield, Mass. The scientific character and value of the contributions that have in the past come from this institution have not heretofore received the general recognition which they undoubtedly deserve.

Textbook on Biology†

A TEXTBOOK on general biology for collegiate students which aims to give a general recapitulation while avoiding too technical considerations. Comparative biology is presented as observed in the animal and vegetable kingdoms, followed by certain practical applications and deductions including the theories of heredity, a discussion of acquired characteristics, theories of evolution, the study of fossils and its application to the theory of adaptation, the geographical distribution and meaning of color, and the effects of environmental changes.

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The Fourth Annual Convention of the American Society of Clinical Pathologists

Just as we go to press news has reached us of the great success of the American Society of Clinical Pathologists meeting held May 20-23 at Philadelphia. Close to 150 members signed the registration book. The enthusiasm was as great and as inspiring as characterized the Rochester conclave a year ago. All of the essayists, save one, who was incapacitated by illness, were present at the meeting to read their respective papers. Needless to say, the papers were of a high scientific character.

The new officers elected are as follows: President, Dr. Frederick Sondern, of New York; President-elect, Dr. William G. Exton, Newark, N. J.; Vice-President, Dr. F. L. Burnett, Boston, Mass.; Secretary-Treasurer, Dr. Ward Burdick, Denver, Colo. Member of the Executive Committee, Dr. Philip Hillkowitz, Denver, Colo. Member Board of Censors, Dr. Geo. Ives, St. Louis, Mo.

Full details of the meeting will appear in the next issue.

CLINICAL AND EXPERIMENTAL

·AN ATTEMPT TO FIND AN ANTIPEPTIC ENZYME IN THE BLOOD*

By Venice D. Keiser, M.D., Rochester, Minnesota

Fellow in Surgery, The Mayo Foundation

MANY clinicians regard peptic ulcer as a local digestive process in the stomach or duodenum. That the stomach does not digest itself is a physiologic phenomenon for which there is little or no explanation. It seems logical, therefore, that any data that might explain why the stomach does not digest itself might also throw some light on the etiology of peptic ulcer.

Digestion in the stomach and duodenum, proximal to the ampulla of Vater, is due to the action of pepsin and hydrochloric acid. If peptic ulcer is a local digestion (autodigestion) of the stomach or duodenum by its own digestive juices, it follows that peptic ulcer may be in some way related to an abnormal pepsin hydrochloric acid mixture, or there may be something abnormal in the wall of the stomach and duodenum that renders these tissues particularly susceptible to the action of the digestive juices in such cases.

The first possibility mentioned, namely, an abnormal pepsin hydrochloric mixture in stomachs with ulcer, has been the subject of much investigation. The textbooks emphasize a high free hydrochloric acid content in ulcer cases, and it is the general inference that in some way the high hydrochloric acid content is responsible for the ulcer. However, it is not uncommon to find patients suffering from ulcer in whose gastric contents free hydrochloric acid is not found. Such findings, even in a few cases, suggest very strongly that the high free hydrochloric acid is probably not the only factor concerned in the formation of peptic ulcer. ·

The pepsin factor in the pepsin hydrochloric acid mixture is one that scarcely lends itself to investigation at this time. It is true that patients with ulcer may have a particularly active pepsin thrown out by the gastric mucosa. However, in the first place, we do not know what pepsin is; it has never been isolated as a chemical entity. We have reason to believe that it is a colloid; we know that it is an enzyme, which, in the presence of free acid brings about proteolytic activity, but our specific knowledge concerning it is deficient. It follows, therefore, that until we know the nature of normal pepsin we cannot possibly point out an abnormal pepsin and assign to such an abnormal product any etiologic relationship to peptic ulcer.

The second possibility is whether or not there is something which renders the stomach and duodenum particularly susceptible to the action of their own digestive juices in ulcer cases. What causes the stomach to digest meat that is ingested as food, and yet does not normally affect the wall of the stomach itself? We are, it seems, considering a problem of much the same nature as the old question of how would it be possible to have a container for a universal solvent. Yet here, there is a solution for that old riddle. The digestive juices will dissolve any meat exposed to them, and yet will not dissolve the walls of the stomach, which are apparently physically and chemically identical to the substance being dissolved. However, the meat ingested as food differs from the flesh of the wall of the stomach, at least in one respect: the one has no blood supply; the other has an intact blood supply which results in a continual bathing of all of its cells with blood and lymph. An excellent experimental review of this question has recently been made by Draagstedt and Vaughn. Is it possible that the circulating blood and lymph protect the stomach and duodenal mucosa from the action of the digestive juices; and is it a lack of such protective substances in the blood stream that results in digestion and ulcer formation in certain cases? In other words, is there a substance in the circulating blood which inhibits peptic activity; and if so, is the lack of, or absence of that substance in any way related to the formation of peptic ulcer? This research was undertaken in an attempt to answer these questions.

REVIEW OF LITERATURE

Since Weinland's work, discussions have appeared in the literature concerning a substance in the blood stream, which is considered to be of the nature of an antienzyme; it is referred to as antipepsin.

Weinland, in working with ascaris worms, was able to extract from them a substance which had a definite pepsin inhibitory effect. He concluded, therefore, that the reason these worms are not digested in the stomach, but are able to pass on and live in the intestine, is because of this pepsin inhibitory substance in their bodies. He suggested further that the reason the stomach does not normally digest itself is because there is probably a similar pepsin inhibitory substance in its walls, or in the circulating blood and lymph.

Sachs injected geese intraperitoneally with increasing doses of pepsin over a period of two and one-half months. He was able to demonstrate the presence of an antipepsin ferment in the blood of these geese. He found, by his method of gelatin liquefaction, that it took twenty times the amount of

pepsin to digest (render fluid) gelatin in the presence of goose blood injected with pepsin, as in the presence of normal goose blood. He explained his findings as due to the development of an antipepsin (a pepsin inhibitory substance) in the blood of geese injected with pepsin, and calls attention to the similarity of this antipepsin to antitoxins, a similarity noted also by Kolmer and Wells.

Oguro likewise investigated the presence of antipepsin in the serums of horse and man. He prefers the use of fibrin carmine in his demonstration, and considers the liberation of the carmine in his series of tubes as evidence of digestion.

Bayliss, however, is unable to demonstrate antienzymes and sums up his opinion as follows: "There is considerable doubt whether true antienzymes exist. Some of the effects described as being due to them are to be accounted for by changes in hydrogen-ion concentration, others by the absorption of the enzymes by colloids."

Hamburger attributes many of the reports of antipepsin as probably due to the inhibitory effect of sodium chloride used in the solutions and manipulations, and he demonstrates that physiologic sodium chloride solution can definitely inhibit peptic activity.

Kolmer sums up the present status of the antiferment question as follows: "According to some investigators antiferments are to be found in large amounts in all normal sera and are probably vitally concerned in life processes in preventing autodigestion. That they may be artificially stimulated is disputed. They certainly never attain the amounts as antitoxins. Various antiferments have been produced according to many investigators but these observations have not been generally confirmed."

METHODS

An investigation was first made of the various methods described in the literature for demonstrating antipepsin. Specimens of blood from normal persons, ulcer-bearing patients, and patients suffering from other diseases, but in whom the diagnosis of peptic ulcer had been eliminated, were used. Blood from normal dogs and from dogs in whom peptic ulcer had been produced by means of Mann's method of draining the alkaline secretion away from the pylorus were also used. The methods investigated depend primarily on a measuring of peptic activity. The antipepsin is estimated by its inhibitory effect on peptic activity.

Liberation of Dye and Protein.—The method employed by Oguro and Hamburger was tried. They use a protein, fibrin, in which carmine is incorporated. A series of tubes is set up, containing increasing amounts of pepsin, constant amounts of hydrochloric acid, and constant amounts of the serums whose antipeptic properties are to be studied. Then flakes of fibrin-carmine are added and the tubes incubated. After incubation, the tubes containing the larger amounts of pepsin are red, due to the liberation of the carmine on digestion of the fibrin; the tubes containing the smaller amounts of pepsin are not so colored because there is no digestion, and hence no liberation of carmine. The reason, therefore, that the pepsin in the colorless tubes had

been bound is due to the presence of antipepsin in the additional serum. The amount of antipepsin present in the serum is then estimated by noting the greatest amount of pepsin that will not produce liberation of the dye in the presence of that serum.

This method was given a thorough trial. Fibrin was prepared by drawing blood in oxalate solution and centrifugalizing out the red cells. The serum or fibrin was then recalcified and clotted, then cut into small bits, the size of a split pea, and soaked in a 0.5 per cent carmine solution. After twenty-four to thirty-six hours the fibrin was thoroughly impregnated with the dye. The particles were then washed with running water for from twenty-four to thirty-six hours. The serum pepsin hydrochloric mixtures were set up in tubes as described by Oguro and Hamburger. The gradation between the colorless tubes and the red tubes was so gradual as not to give any definite idea of an end point. Further, it was not uncommon to get a liberation of the dye in control tubes in which we had added simply hydrochloric acid and no pepsin. This method, therefore, was not applicable to the problem.

Gelatin Liquefaction.—The gelatin liquefaction method of Sachs was investigated. A series of tubes of gelatin solutions from 3 to 18 per cent were set up. These were liquefied and then allowed to stand until the thermometer within the tube registered the same temperature as a thermometer on the work table, thus making sure that the tubes had come down to room temperature. It was noted that when both thermometers registered 22.2° C., the 9 per cent gelatin was solid; the 7.2 per cent gelatin was solid but quivered; the gelatin solutions less than 7 per cent in strength were all fluid. This rack was allowed to stand three hours longer, and then the solutions were observed again. The two thermometers still registered 22.2° C., but at this time the 6 and 5 per cent solutions which were fluid on the first observation had become solid. It seemed, therefore, that gelatin was not suitable as an indicator of digestion, as its state of fluidity or solidity seemed to depend not only on the temperature, but also on other factors. Since it was not known whether a solution of undigested gelatin of a given per cent and at a given temperature would jell or be fluid, the state of digestion could certainly not be estimated by its fluid or solid state.

Mett Tube Principle.—An attempt was made at this point to use the old Mett tube principle of measuring digestion. It was apparent that for this problem Mett tubes would not be sufficiently accurate unless they were more accurately made and standardized than those usually employed in experiments on digestion. Accordingly the Mett tube principle was used, but standardized in a different manner, namely, a measured quantity (0.05 c.c.) of coagulable protein was placed in the bottom of Wassermann tubes. Blood serum from various sources and egg white were used. This protein was carefully shaken to the bottom of the tube, and then coagulated by placing the rack of tubes in boiling water for three minutes. After coagulation, increasing amounts of pepsin solution (constant quantities of serum being examined) and hydrochloric acid were added. The tubes, after digesting in the incubator, were turned upside down. It was hoped that complete digestion would be shown by absence of the coagulated serum, or egg white, in the

bottom of the tube, and incomplete or no digestion by the presence of this coagulated protein in the tube. However, this method likewise proved inaccurate, inasmuch as very often the undigested protein could be seen to slip away from the bottom of the tube. The digestion mixture was turbid, and the question continually arose as to whether or not the turbid tubes near the end point were not due to undigested protein that had fallen away from the bottom of the tubes. Further, although the quantity of protein was constant, within limits of experimental error, the surface areas were not constant and this is a possible cause for error.

Physical and Chemical Methods.—An investigation was made into the physical and chemical methods described in the literature for measuring digestion, with the view that, once the proper index of digestion was found, it could readily be applied to the measuring of an antipepsin by the antipepsin's inhibitory effect on peptic digestion, as shown by the given index of digestion. At our disposal were: (1) rotation of polarized light, 10; (2) changes in electrical conductivity, 11; (3) changes in index of refraction of light, and (4) determination of nonprotein nitrogen, 12.

Therefore, a series of tubes were set up containing blood serum, 1 c.c.; pepsin, 1 c.c. (1:100 solution), and one-tenth normal hydrochloric acid, 8 c.c. The serums used were from cases of ulcer, normal controls, and dogs injected with pepsin. It was considered unnecessary to add protein for the pepsin to digest, since the serum being examined contained 6 to 8 per cent protein. Polariscopic and refractometer readings were taken on these solutions, before and after digestion. The differences in readings before and after digestion were so small, if any, that they were entirely within the limits of error of the apparatus.

Similar tubes were set up, as in the preceding experiment, and the nonprotein nitrogen was determined before and after digestion. There was no constant difference in the nonprotein nitrogen, before and after digestion, that designated ulcer or normal bloods in the group.

<center>DISCUSSION</center>

Throughout this work, as in the beginning, the question continually arose as to whether or not the substance being sought did, or did not actually exist. The question is still an open one. The methods described in the literature as demonstrating an antipepsin were used, and were found to be either not sufficiently accurate for the problem, or entirely incapable of application. The technical difficulties are numerous. There are many factors which have to be constantly controlled. In the first place aseptic technic is desirable, although difficult to carry out. The pepsin solution must be sterile, and kept sterile by passage through a Berkefeld filter. The pepsin used was regularly contaminated by a Gram-positive proteolytic bacillus, not unlike B. subtilis, but the organism failed to give the characteristic sugar reactions of B. subtilis. The conditions of digestion must be accurately controlled. The hydrogen-ion concentration plays an important part. It was practically impossible to keep this factor constant, except within rather wide limits, owing to the fact that

the digestion reaction must necessarily go on in the presence of the examined serum. These serums all vary in their hydrogen-ion concentration.

There are other factors such as, (1) temperature of digestion mixture, (2) length of time digestion is permitted to proceed, (3) total volume of the digestion mixture, in order to dilute digestion products which inhibit digestion, (4) the strength of the pepsin solution, and (5) the length of time during which the pepsin and serum are allowed to remain in contact with each other before adding the hydrochloric acid. All of these factors play a part, and it may be that an ideal combination of all of them will lead to findings other than our own.

The paramount difficulty is the method of measuring digestion. The methods of liberation of a dye from protein and of gelatin liquefaction, in our hands, have not proved applicable to the problem. Measurement of changes in the physical properties of the digestion are of no avail. The measurement of nonprotein nitrogen as an index of digestion seemed promising, but we were at once confronted with the choice of a protein precipitant. It is possible that the precipitants used, that is, sodium tungstate, trichloracetic acid, and sulphosalicylic acid, carried with them certain digestion products which resulted in our variable findings.

·At any rate, it is probable that the antipepsin spoken of in the literature has not been demonstrated. The methods described in the literature as applicable to their demonstration do not prove the presence of such a substance, and no doubt an antipepsin will be proved to be present or absent only when more accurate methods for its detection have been evolved.

SUMMARY

In view of the fact that peptic ulcer is considered a local autodigestion of the stomach, an investigation was made of the protective antienzyme theory of gastric protection. This theory, if it explains lack of autodigestion of the stomach, should likewise be applicable to the etiology of peptic ulcer. The methods described in the literature as demonstrating an antipepsin were used and found to be inaccurate. These methods neither proved nor disproved the presence or absence of such a substance. Antipepsin may or may not exist, and it may or may not have a bearing on the etiology of peptic ulcer. More accurate methods must be devised before this question of the relationship between antipepsin and peptic ulcer can be answered.

REFERENCES

1Bayliss, W. M.: Principles of General Physiology, New York, ed. 3, 1920, Longmans, Green and Co., pp. 316, 331.

2Draagstedt, L. R., and Vaughn, A. M.: Gastric Ulcer Studies, Arch. Surg., 1924, viii, 791-810.

3Hamburger, W. M.: The Inactivation of Pepsin by Sodium Chloride: Its Clinical Significance, Arch. Int. Med., 1915, xvi, 356-362.

4Kolmer, J. A.: Textbook on Infection, Immunity and Specific Therapy, with Special Reference to Immunologic Technic, Philadelphia, ed. 2, 1917, W. B. Saunders Co., pp. 254-256.

5Mann, F. C., and Williamson, C. S.: The Experimental Production of Peptic Ulcer, Ann. Surg., 1923, lxxvii, 409-422.

6Oguro, Y.: Ueber eine Methode zum quantitativen Nachweis des Antipepsins im Serum, Biochem. Ztschr. 1909, xxii, 266-277.

[7]Sachs, H.: Ueber Antipepsine, Fortschr. der Med., 1902, xx, 425-428.

[8]Schütz, Emil: Eine Methode zur Bestimmung der relativen Pepsinmenge, Ztschr. f. physiol. Chem., 1885, ix, 577-590.

[9]Schütz, Julius: Zur Kenntniss der quantitativen Pepsinwirkung, Ztschr. f. physiol. Chem., 1900, xxx, 1-14.

[10]Sjöqvist, J.: Physiologisch-chemische Beobachtungen über Salzsäure, Skandinav. Arch. f. Phys., 1893-1895, v, p. 317.

[11]Weinland, E.: Zur Frage weshalb die Wand von Magen und Darm während des Lebens durch die proteolytischen Fermente nicht angegriffen wird, Ztschr. f. Biol., 1902, xliv, 45-60.

[12]Wells, H. G.: Chemical pathology, Philadelphia, ed. 4, 1920, W. B. Saunders Co., p. 50.

A STUDY OF INSULIN ACTION*

By Edward C. Mason, M.D., and C. W. Matthew, Ph.C., Ann Arbor, Mich.

SINCE the introduction of insulin there has developed a very comprehensive literature covering its preparation and clinical application. However, little has developed as to how insulin enables the individual to utilize sugar. This latter point has been recently emphasized in a brief report made by Banting[1] on the "Pharmacologic Action of Insulin."

Previous studies, attempting to determine the mechanism of blood-sugar reduction by insulin, have been along four general lines.

First: The action of insulin on the blood—Eadie, Macleod, and Noble.[2]

Second: The action of insulin in the blood stream with the presence of extracts of glands of internal and external secretions—*a.* adrenalin: Ross and Davis[3] and Banting, Best, Collip, Macleod and Noble[4]; *b.* pituitary extract: Burns[5] and Olmstead and Logan[6]; *c.* parathyroid extract: Winter and Smith[7] and Forrest[8]; *d.* trypsin: Epstein, Rosenthal et al.[9]

Third: The action of insulin following the removal of viscera—*a.* thyroidectomy: Bodansky,[10] Ducheneau,[11] and Burns and Marks[12]; *b.* adrenalectomy: Stewart and Rogoff[13]; *c.* hepatectomy: Mann and Magath[14]; *d.* decapitated and eviscerated cats· Burns and Dale.[15]

Fourth: The action of insulin in perfusion experiments—*a.* isolated heart: Maclean and Smedley,[16] Knowlton and Starling,[17] Patterson and Starling,[18] Clarke,[19] Hepburn and Latchford,[20] Burns and Dale.[15]

Although these methods have not been successful in determining the exact mechanism of insulin action, there have been discovered measures whereby the lowering of blood sugar by insulin may be either completely neutralized or greatly enhanced

Apparently the mechanism of insulin action must be considered as being of twofold: its *primary* action and its *secondary* action. The primary action covers only the mechanism of blood-sugar reduction while the secondary action is observed in the convulsions which accompany the hypoglycemia of excessive doses of insulin. In the present work we have attempted to study only the primary action, as the secondary action has been carefully studied by Olmstead and Logan[6] and Cannon, McIver and Bliss.[21]

In reviewing the literature on insulin, we were impressed with the possibility that the rôle of the nerve mechanism in insulin action had not been fully considered. We have therefore made a study of this phase of the problem, thinking it might yield some additional information on the subject. Our object has been to stimulate or paralyze portions of the autonomic and central nervous systems and follow such procedures with insulin administration and blood-sugar determinations.

METHOD

We have used only unanesthetized animals as it is a well-established fact that anesthesia has a marked effect on the metabolism of body sugar. In our studies involving the destruction of the central nervous system, we gave preliminary ether anesthesia but discontinued it as soon as possible. The insulin used was that of Eli Lilly Co., and all administrations were made intravenously, the dose usually being four units per kilo in both the rabbit and dog. Blood samples were taken before the administration of insulin and usually at periods of one-half hour and one and one-half hours following. The blood was obtained from the marginal ear veins in the rabbit (2 c.c. samples) and from the femoral vein in the dog (5 c.c. samples). The blood-sugar determinations were made according to the method of Folin and Wu.[22]

STUDIES ON THE SYMPATHETIC SYSTEM

It has been demonstrated by Banting, Best, Collip, Macleod, and Noble[4] that there exists an antagonism between adrenalin and insulin in their action on blood sugar. This observation has been repeated by other workers and in order to standardize our technic, we have repeated similar experiments using both the dog and the rabbit. Two of such experiments are briefly summarized in Table I.

TABLE I

Dog 1. Male, 15 Kilo. Not Fed in Previous Twenty-Four Hours

	TIME	BLOOD SUGAR
Normal Blood Sugar		94.3
Insulin 60 units	7.53	
Blood Sugar	8.32	66.2
Blood Sugar	9.15	53.7
2 c.c. Adrenalin (1-10000)	9.22	
Blood Sugar		62.5
Rabbit 1. 2 Kilo.	9.57	117.6
Insulin 8 units	10.12	
Blood Sugar	10.50	78.8
Blood Sugar	11.42	76.1
1 c.c. Adrenalin (1-10000)	11.45	
Blood Sugar	11.55	92.9

The power of adrenalin to thus counteract the hypoglycemia of insulin suggests the possibility that the two drugs may be antagonistic physiologically. Therefore, in order to test the rôle of the sympathetic system in the production of hypoglycemia by insulin, we have attempted to paralyze the sympathetic ganglion by use of nicotine and lobeline and follow their administration by insulin. Table II briefly outlines the results.

TABLE II

RABBIT 5.   3.64 KILO

	TIME	BLOOD SUGAR
0.5 c.c. Nicotine 0.5% (nearly died)	3.21	
0.25 c.c. Nicotine 0.5%	3.30	
Blood Sugar	3.32	150.4
Insulin 15 units	3.37	
Blood Sugar	4.10	44.5
Blood Sugar	5.10	47.8
DOG 5. 17 KILO. NOT FED IN PREVIOUS TWENTY-FOUR HOURS		
Blood Sugar	9.35	112.2
Lobeline 0.5 c.c. (0.5 mg./c.c.)	9.40	
Lobeline 1.0 (0.5 mg./c.c.)	9.45	
Insulin 70 units	9.52	
Lobeline 1.5 c.c. (0.5 mg./c.c.)	9.55	(definite reaction)
Blood Sugar	10.27	64.5
Blood Sugar	11.22	58.0

From the results shown in Table II it is suggested that the sympathetic system is not primarily involved in the production of hypoglycemia by insulin. We have confirmed these results with varying doses of lobeline and nicotine in combination with atropine and partial destruction on the central nervous system.

STUDIES ON THE PARASYMPATHETIC SYSTEM

Garrelton and Santenoise[23] have noted that insulin is a powerful excitant of the vagus, the oculocardiac reflex being augmented, the heart and respiration slowed, and the blood pressure falls. The susceptibility to shock increases. They found that such a picture was independent of blood-sugar decrease, as previous injections of glucose did not influence the results. They suggest that albumin in the insulin may modify the results and that certain hypovagotonic animals do not respond. Houssay and Lewis[24] have observed that the blood-sugar increase which accompanies morphine may be hindered by section of the splanchnics, but comes into play again if the vagi are cut. We have observed several animals which have received atropine before insulin and Table III expresses the results.

TABLE III

DOG 4.   FEMALE, 17 KILO.   NOT FED IN PREVIOUS TWENTY-ONE HOURS

	TIME	BLOOD SUGAR
Blood Sugar	9.20	84.8
Atropine 3 mg.	9.29	
Atropine 1 mg.	9.33	
Blood Sugar	9.38	84.1
Insulin 68 units	9.42	
Blood Sugar	10.15	61.7
Blood Sugar	11.30	57.4
RABBIT 5. (PREVIOUSLY REPORTED.) 3.64 KILO.		
Atropine 5 mg.	3.15	
Atropine 7.5 mg.	3.17	
Nicotine		
Blood Sugar	3.32	150.4
Insulin 15 units	3.37	
Blood Sugar	4.10	44.5
Blood Sugar	5.10	47.8

We have confirmed these results by using atropine with other drugs and from the findings, it appears that insulin acts as effectively after the animal has received large doses of atropine.

PITUITARY EXTRACT

Burns,[5] in his splendid studies on insulin and pituitrin, has demonstrated that subcutaneous injections of the extract of the posterior lobe of the pituitary gland given simultaneously with injections of insulin, diminished or abolished the fall of blood sugar normally produced by the insulin. He has further shown that pituitary extract removes the symptoms of hypoglycemia convulsions causing a rapid elevation of blood sugar. We have been able to confirm his observations as illustrated in Table IV.

TABLE IV

DOG 2.   15 KILO.   NOT FED IN PREVIOUS EIGHTEEN HOURS

	TIME	BLOOD SUGAR
Blood Sugar	9.25	74.7
Insulin 60 units	9.30	
Blood Sugar	10.5	33.7
Blood Sugar	11.10	33.2
Pituitrin 1 c.c.	11.16	
Blood Sugar	11.32	58.5
RABBIT 2. 2.27 KILO		
Blood Sugar	9.45	108.1
Insulin 9 units	9.51	
Blood Sugar	10.22	44.8
Blood Sugar	11.27	43.7
Pituitrin 0.3 c.c.	11.29	
Blood Sugar	11.40	81.3

It is impossible to explain how adrenalin and pituitrin acting on any single known peripheral nerve mechanism can counteract the hypoglycemic action of insulin. Burns has called attention to the fact that although both adrenalin and pituitrin produce a hyperglycemia, adrenalin produces a greater and more prolonged hyperglycemia and the antagonistic action of pituitrin to insulin hypoglycemia is more extensive, more persistent, and more rapid in its action.

STUDIES ON CENTRAL NERVOUS SYSTEM

The observations of Cushing[25] and his collaborators serve to show that there is a definite relation between carbohydrate metabolism and the pituitary body. Clinically and experimentally they noted that hypophyseal deficiency was accompanied by "an acquired high tolerance for sugars, with the resultant accumulation of fat." Borchardt[26] found that infundibular extract caused a rise in blood sugar. However, such an increase was denied by Franchini.[27] Later, Cushing showed that the intravenous administration of the extract was "almost without exception" accompanied by hyperglycemia.

In attempting to study insulin convulsions in decerebrated cats Olmstead and Logan were unable to cause a lowering of blood sugar in the decerebrated animals to the level necessary to produce convulsions in the rabbit. They then attempted to determine whether such a high blood sugar in the decere-

brated cat was not attributable to the action of the pituitary body. After considerable work on decerebrated cats with the pituitary body intact, and with the pituitary body removed, they concluded that "decerebrated cats with the pituitary body intact maintain a high blood-sugar level which is not materially reduced by insulin." Analysis of their results indicate that both the animals with the pituitary body intact and with the pituitary removed responded to the administration of insulin, the amount of blood-sugar lowering being essentially the same in the two conditions. However, in the animals with the intact pituitary the blood sugar was at a higher level at the time of the insulin administration and therefore the resulting blood sugar did not reach so low a level.

Burns and Dale[15] have recently published experimental results which have a very definite relation to this phase of our work. They observed that in the decapitated and eviscerated cat, with constant infusion of dextrose, insulin produces the characteristic fall of blood sugar.

In our studies involving the central nervous system we have attempted to avoid asphyxiation, for Yamakani[28] has demonstrated that clamping the trachea of the rabbit would increase the blood sugar from normal, i.e., about 0.120 per cent to 0.225 per cent, such increase occurring in five minutes. We have not attempted any studies which would necessitate the intact respiratory center but instead have used artificial respiration which could be accurately controlled and thereby have avoided any changes which might be caused by respiratory stimulation or depression.

In destroying the central nervous system we have used essentially the technic described by Jackson.[29] We have studied both the action of insulin after destruction of the brain and cord and also with the addition of atropine, lobeline and nicotine. Table V represents the results obtained after pithing an animal's brain and cord.

TABLE V

DOG 12.7 KILO. FED TWENTY-FOUR HOURS PREVIOUSLY

	TIME	BLOOD SUGAR
Blood Sugar (under ether)	11.00	180.1
Pithed	11.07	
Blood Sugar	11.17	192.2
Blood Sugar	11.27	168.5
Insulin 70 units	11.31	
Blood Sugar	12.01	74.1
Blood Sugar	12.18	49.0
Insulin 80 units (Stearn's)	12.21	
Blood Sugar	12.53	63.7

Comment: Blood sugar apparently increased following the second injection of insulin.

In the second animal of this series, we did not only pith brain and cord but in addition gave 4 milligrams of atropine in divided doses and also 10 milligrams of nicotine in divided doses. Fig. 1-a is a record obtained from the animal after it had been given atropine, nicotine and a pithed brain and cord. The record was made during the period which insulin action was studied. It will be noted that the animal received two injections of insulin—each representing 100 units, yet there was no reduction of blood sugar but

Dog — 21 K — After Atropine and Nicotine
Pithed Brain and Cord

Fig. 1-a.

Dog — 21.2. K — After Atropine, Lobeline
Pithed Brain and Cord
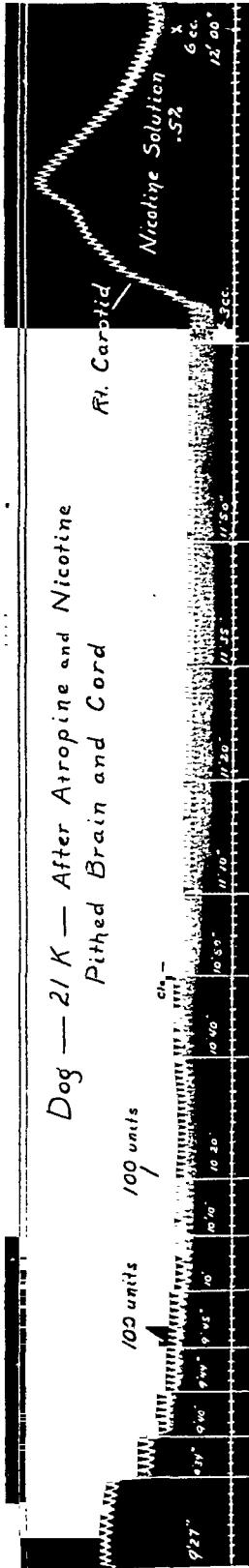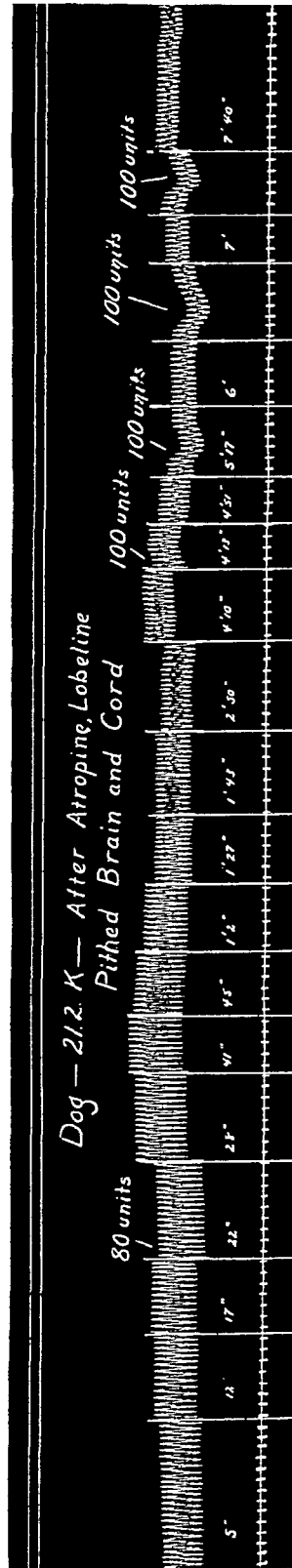
Fig. 1-b.

TABLE VI

Dog 21 Kilo. Not Fed During Previous Thirty-Six Hours

	TIME	BLOOD SUGAR
Blood Sugar	8.45	97.1
Atropine 4 mg. (Divided doses)	9.00	
Nicotine 2 c.c. 0.5% (Divided)	9.15	
Pithed	9.27	
Blood Sugar	9.35	177.0
Blood Sugar	9.42	200.
Insulin 100 units	9.45	
Blood Sugar	9.55	256.4
Blood Sugar	10.15	285.7
Insulin 100 units	10.18	
Blood Sugar	10.28	317.5
Blood Sugar	10.48	307.7

instead there was a gradual increase. A sample of this same insulin was returned to Eli Lilly and Co., who reported it to be of original potency. It will also be noted that toward the end of the record the animal received a large dose of nicotine which caused a definite blood pressure response. Such a result suggests that the original dose of nicotine was not sufficient to cause
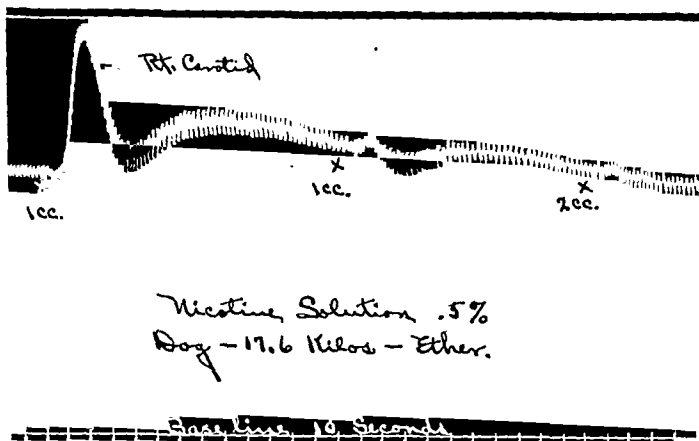
Fig. 2.

paralysis or that its action had worn off during the experiment. Table VI is a summary of procedure and results obtained.

We have repeated the experiment explained in Table VI but using considerably more nicotine. Fig. 2 is the record obtained during the administration of nicotine, the animal having previously received 4 mg. of atropine sulphate. It will be noted that following the first injection of 1 c.c. of nicotine

solution there was a prompt and pronounced rise in blood pressure, the second dose of 1 c.c. of nicotine solution gave little response, while the third injection of 2 c.c. caused practically no change in blood pressure. Following the administration of nicotine the animal was pithed completely, brain and cord. The data presented in Table VII gives a brief record of procedure and results obtained.

TABLE VII

DOG 17.6 KILO. NOT FED DURING PREVIOUS TWENTY-FOUR HOURS

	TIME	BLOOD SUGAR
Blood Sugar (Under Ether)	2.30	123.5
Atropine 1 c.c. (1 mg.)	2.33	
Atropine 1 c.c.	2.35	
Atropine 2 c.c.	2.36	
Nicotine 1 c.c. (0.5%)	2.39	
Nicotine 1 c.c.	2.41	
Nicotine 2 c.c.	2.42	
Blood Sugar	2.44	159.4
Pithed	2.55	
Blood Sugar	3.08	119.8
Repithed	3.11	
Blood Sugar	3.18	111.6
Insulin 100 units	3.25	
Blood Sugar	3.55	84.1
B'ood Sugar	4.25	60.6
Blood Sugar	4.55	39.4

It will be observed that during the administration of the atropine and nicotine the blood sugar rose from 123.5 mg. to 159.4 mg., also that this animal, although receiving a much larger dose of nicotine, still responded to insulin administration. These observations suggested to us the possibility that probably nicotine had exerted only a stimulating effect in the previous experiment, and through stimulation of the sympathetic system had left the blood sugar high even in the presence of excess insulin. (We repithed the animal because of some twitching about the mouth which suggested incom-

TABLE VIII

DOG 17 KILO. NOT FED PREVIOUS FORTY-EIGHT HOURS

	TIME	BLOOD SUGAR
Blood Sugar (Under Ether)	10.20	119.1
Blood Sugar (Under Ether)	10.33	137.4
Pithed	10.50	
Atropine 1 mg.	10.52	
Blood Sugar	11.05	196.1
Blood Sugar	11.20	116.3
Blood Sugar	11.35	122.0
Nicotine 2/3 c.c. (0.5%)	11.39	
Blood Sugar	11.45	114.9
Nicotine 0.5 c.c. (0.5%)	11.52	
Insulin 95 units		
Blood Sugar	12.02	111.7
Nicotine 0.5 c.c. (0.5%)	12.05	
Blood Sugar	12.12	95.2
Nicotine 0.5 c.c. (0.5%)	12.15	
Blood Sugar	12.22	87.3
Blood Sugar	12.52	71.4
Artificial respiration discontinued	1.45	

plete pithing. However, we later concluded that such movements were caused by the administration of nicotine.)

Assuming that nicotine in small doses might exert a stimulating effect on the sympathetic system and thereby maintain a high blood sugar, we attempted the following experiment to determine whether such an action was responsible for some of our results. We also desired to observe the variations in blood sugar of an animal pithed brain and cord. The results of our observations are recorded in Table VIII.

On account of the extreme action of the vagus, following the pithing of
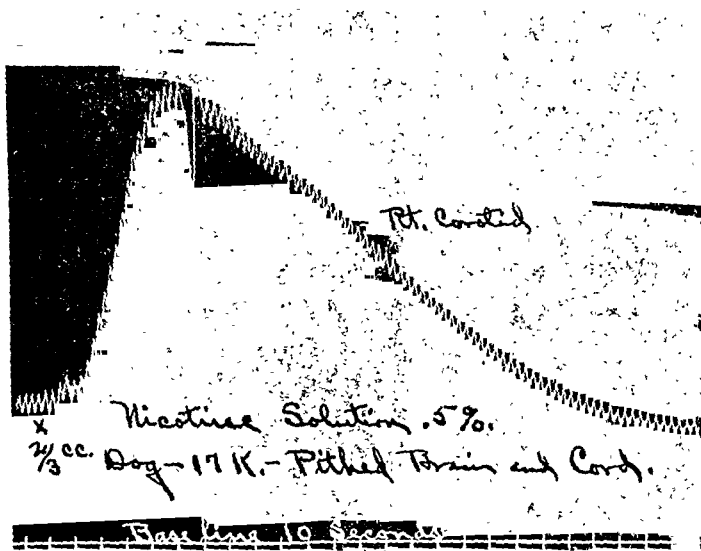
FIg. 3.

the animal, it was thought necessary to give atropine. One milligram was sufficient to free the heart from what appeared to be a dangerous inhibition. The blood sugar rose, following pithing, to 196.1 mg. and during the following thirty minutes appeared to reach a rather constant level of about 122 mg.

After the blood sugar had attained such a constant level the animal received ⅔ c.c. nicotine solution 0.5 per cent. Fig. 3 is presented to show the blood pressure response to the nicotine administration. The blood sugar, following the return of the blood pressure to normal, was essentially the same as before the administration of the nicotine. The animal then received 0.5 c.c. of nicotine solution and simultaneously 95 units of insulin. The blood
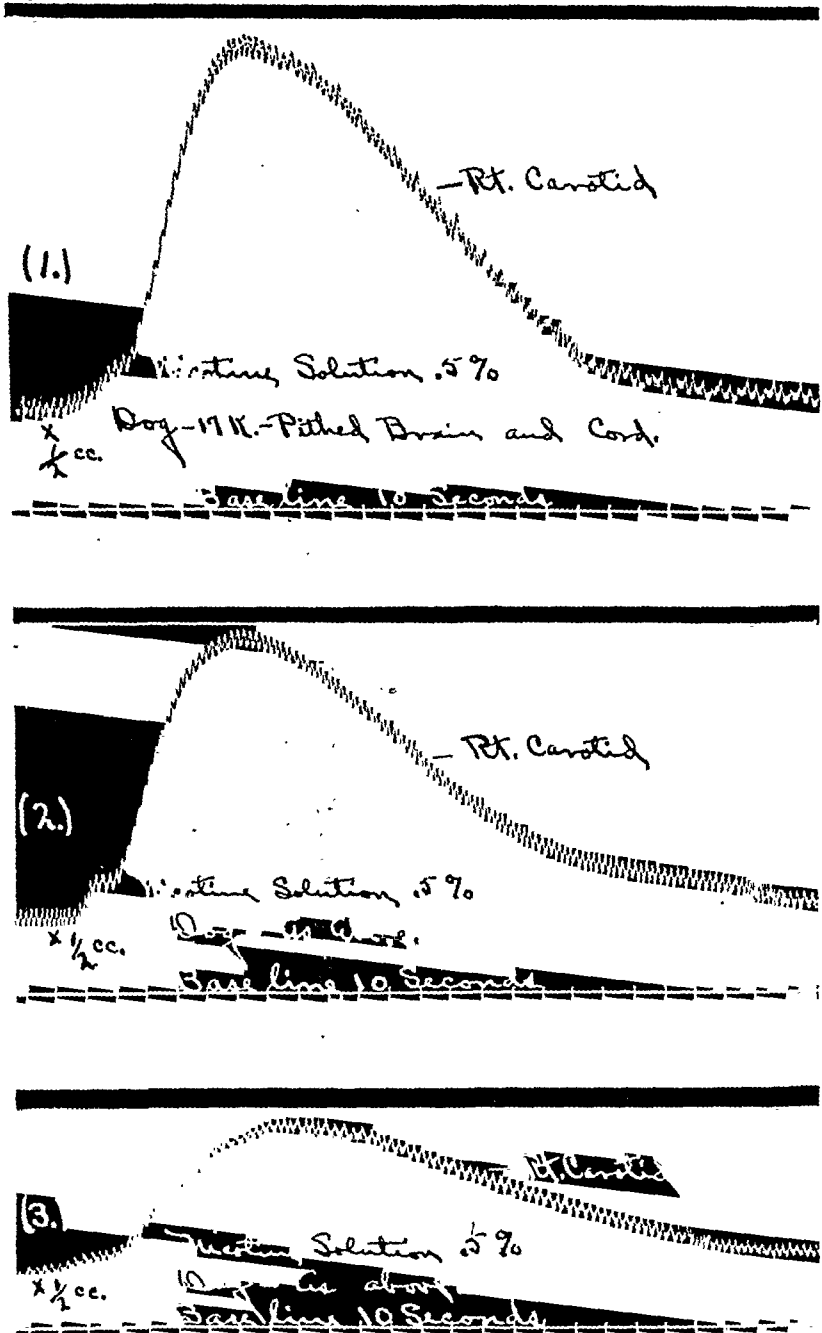
Fig. 4.

sugar was determined each ten minutes and 0.5 c.c. nicotine solution was also administered each ten minutes. Fig. 4 shows the result of such administrations—the response becoming less with each injection, yet each produced a definite increase in blood pressure. The results of the blood-sugar determinations accompanying the insulin-nicotine administration are presented in Table VIII.

Table IX is the summary of the data obtained from an animal which had received insulin following the administration of atropine and lobeline. The blood pressure record of the animal is presented in Fig. 1-b.

TABLE IX

Dog 21.2 Kilo.   Spleen Previously Removed

	TIME	BLOOD SUGAR
Atropine 1 c.c. (1 mg. per c.c.)	3.40	
Lobeline 8 c.c. divided doses (0.5 mg. per c.c.)	3.50	
Pithed brain and cord	4.05	
Blood Sugar	4.10	208.1
Blood Sugar	4.20	208.1
Insulin 80 units	4.22	
Blood Sugar	4.32	246.9
Blood Sugar	4.52	188.7
Blood Sugar	5.52	56.5
Blood Sugar	7.02	33.5
Insulin 100 units	8.18	
Blood Sugar	8.51	100. (Arterial Blood)
Insulin 100 units	9.22	
Insulin 100 units	10.09	
Insulin 100 units	11.07	
Stopped artificial respiration	11.50	

Comment: Blood sugar rose immediately following the second administration of insulin. (The animal received several times the usual toxic dose of insulin without any apparent toxic results.)

CONCLUSIONS

1. Insulin produces blood-sugar lowering after the following procedures: (*a*) the destruction of the central nervous system, (*b*) the administration of atropine in doses sufficiently great to paralyze the parasympathetic endings, and (*c*) the administration of nicotine and lobeline in doses which stimulate and paralyze the sympathetic ganglia.

2. One animal of the series, which was pithed brain and cord and which also received atropine and nicotine, showed a marked increase in blood sugar following the administration of excessive doses of insulin.

3. Small doses of nicotine which stimulate the sympathetic system do not counteract the blood-sugar lowering produced by insulin.

4. Insulin apparently acts independently of the central nervous system, the sympathetic nervous system, and the parasympathetic nervous system.

REFERENCES

[1] Banting: Jour. Am. Med. Assn., 1924, lxxxiii, 1078.
[2] Eadie, Macleod, and Nob'e: Am. Jour. Physiol., 1923, lxv, 462.
[3] Ross and Davis: Jour. Pharmacol. and Exper. Therap., 1922, xx, 121.
[4] Banting, Best, Collip, Macleod, and Noble: Am. Jour. Physiol., 1922, lxii, 559.
[5] Burns: Jour. Physiol., 1923, lvii, 318.

⁶Olmstead and Logan: Am. Jour. Physiol., 1923, lxvi, 437.
⁷Winter and Smith: Jour. Physiol., 1923, lviii, 108.
⁸Forrest: Brit. Med. Jour., 1923, ii, 916.
⁹Epstein, Rosenthal, et al: Am. Jour. Physiol., 1924, lxx, 225; 1925, lxxi, 216.
¹⁰Bodansky: Proc. Soc. Exper. Biol. and Med., 1922-23, xx, 538.
¹¹Ducheneau: Compt. Soc. de biol., 1924, xc, 248.
¹²Burns and Marks: Jour. Physiol., 1924, lix, Physiol. Soc., viii.
¹³Stewart and Rogoff: Am. Jour. Physiol.; 1923, lxv, 342.
¹⁴Mann and Magath: Am. Jour. Physiol., 1923, lxv, 403.
¹⁵Burns and Dale: Jour. Physiol., 1924, lix, 164.
¹⁶Maclean and Smedley: Jour. Physiol., 1912, xlv, 21.
¹⁷Knowlton and Starling: Jour. Physiol., 1912, xlv, 146.
¹⁸Patterson and Starling: Ibid., 1913, xlvii, 137.
¹⁹Clarke: Johns Hopkins Hosp. Rep., xviii, 229.
²⁰Hepburn and Latchford: Am. Jour. Physiol., 1922, lxii, 177.
²¹Cannon, McIver, and Bliss: Am. Jour. Physiol., 1924, lxix, 46.
²²Folin and Wu: Jour. Biol. Chem., 1920, xli, 367.
²³Garrelon and Santenoise: Compt. rend. Soc. de biol., 1924, xc, 470.
²⁴Houssay and Lewis: Compt. rend Soc. de biol., 1923, lxxxix, 1120.
²⁵Cushing: The Pituitary Body, Philadelphia, 1910, J. B. Lippincott Co.
²⁶Borchardt: Ztschr. f. klin. Med., 1908, lxvi, 332.
²⁷Franchini: Berl. klin. Wchnschr., 1910, xlvii, 613.
²⁸Yamakani: Am. Jour. Physiol., 1919, l, 177.
²⁹Jackson: Experimental Pharmacology, St. Louis, 1917, C. V. Mosby Co., p. 291.

FINANCING CLINICAL PATHOLOGY IN THE MODERN HOSPITAL*

By Ward Burdick, M.D., Denver, Colorado

IT is again my privilege, as the representative of the American Society of Clinical Pathologists, to express the appreciation of the courtesy in extending the opportunity to present a subject, in the endeavor to effect a reconciliation in what has probably been one of the most difficult problems, which has confronted the advocates of hospital standardization up to the present time.

It is the purpose of this presentation to represent a composite of the opinions derived from the fellows of the Society as the result of a questionnaire.

The concluding paragraph of the communication presented a year ago before the Congress at Chicago, may be quoted: "In closing may I suggest the appointment of a committee by the American College of Surgeons to cooperate with a similar committee from the American Society of Clinical Pathologists, to formulate a plan of financing the department of clinical pathology in the hospital?" In the absence of action proceeding from this suggestion, it is deemed proper to again present the matter for consideration, with the hope that an agreeable plan might be developed. The subject is one which is best approached with due consideration of the five chief participants, or those principally concerned, namely, (1) The American College of Surgeons, (2) The Hospital, (3) The Attending Physician, (4) The Patient, and (5) The Clinical Pathologist.

The American College of Surgeons is generally regarded as a most powerful constructive agency operating in the field of medicine. Under its influence the chaff in surgery is being winnowed out, unnecessary operations eliminated

and illegal ones curtailed. Hospitals are transformed from mere boarding houses, to institutions of science. Uniformity in the recording of cases and the bringing together of staff groups at regular intervals for the interchange of views and the scientific discussion of cases, has been accomplished. As most pertinent to this presentation, however, may be mentioned "The Minimum Standard" laid down by the college, which reads: "That diagnostic and therapeutic facilities under competent supervision be available for the study, diagnosis, and treatment of patients, these to include at least a clinical laboratory providing chemical, bacteriological, serological and pathological services." Thus, the seed of scientific medicine is likewise being implanted, and valuable methods of diagnosis, which come within the realm of clinical pathology are now advocated as routine measures, to be applied to all patients who enter the hospitals.

"Competent supervision" is interpreted by the College as meaning a clinical pathologist; and the laboratory procedures which are suggested as a minimum routine for all patients are, qualitative analysis of the urine, cytologic analysis of the blood, and the histologic examination of all tissues, apparently diseased, removed at operation. This, in the opinion of E. W. Willetts of Pittsburg, "is an irreducible minimum," and, adds R. Ottenberg of New York City, "is not too much to demand of a standard hospital," since, to quote from Kano Ikedo of Minneapolis, "The first and primary function of the hospital laboratory is to aid in correct diagnosis and to guide in proper treatment by furnishing to the attending physician certain negative and positive findings"; while H. C. Sweaney of Chicago, voicing the consensus of opinion, believes "that these requirements should be broadened," for, continues the doctor, "the benefits to be derived are too well known, especially when a thoroughly trained man takes the work in hand."

The impossibility for all standardized hospitals to obtain a full-time clinical pathologist may be gleaned from the fact that, according to estimates, there are less than eight hundred clinical pathologists available at the present time; while in the United States there are 806 hospitals with a bed capacity of 100 and over, to say nothing of the numerous smaller ones. Futhermore, many clinical pathologists are occupied with their private laboratories and are hence reluctant to accept full-time positions, especially in view of the uncertain economic state of hospital service. However, Frank W. Hartman of Detroit, is impressed, "that competent supervision would not necessarily mean that the clinical pathologist must be a full-time member of the Hospital Staff," for, says A. H. Sanford of the Mayo Clinic: "His time might be divided with a minimum of two hours a day to be spent in each laboratory" and, adds T. H. Boughton of Akron, Ohio: "A small hospital can usually arrange for such service by cooperation with a larger one, if the expense is too great to maintain a completely equipped laboratory and staff. We have this arrangement with three such hospitals, one in Akron and two outside." Voicing the same sentiment which, in view of the scarcity of workers in this field, is the consensus of opinion of the clinical pathologists, F. C. Narr of Kansas City states

that "For smaller hospitals, I can see no objection to having one pathologist supervising the work of two or three."

As the question in hand pertains to the financing of this new department, our attention will be directed to the manner in which the subject is viewed by the hospitals.

Hospitals generally have adopted the ideals as suggested by the American College of Surgeons. One cannot view the efforts of these institutions to comply with the standards without feeling a sense of pride and satisfaction. By their efforts the standard of medicine is being elevated, in spite of the fact that they have encountered stubborn opposition.

These institutions have had to meet the complaints of patients against the extra charge for routine laboratory work. This has been largely due to the apathetic attitude of some physicians and surgeons toward standardization. But, says V. L. Andrews of Pittsburg: "The public generally is becoming educated to the fact that a laboratory is a great help to the doctor and many of them are demanding laboratory work and are going to doctors, by preference, who have this work done." And, observes Frederick E. Sondern of New York City: "Patients complain against charges for laboratory work only when the attending physician neglects to inform them in advance that this is a necessary expense, just as his fee is a necessary expense." Regarding the fear that has been expressed in some quarters that insistence upon this added expense is resulting in the opening of small nonstandardized hospitals to the detriment of the larger ones which are trying to maintain the standards; Harry J. Corper of Denver, in agreement with the general opinion, says: "There is just as much justification in the hospital's opposition to doing good clinical pathology on the basis of the patients complaining against the extra charge for laboratory work, as there would be on the basis of the patients complaining against the surgeon's or internist's fee—true, some patients complain about both, and poorer grade hospitals will crop up now and then in violation of standards, but we notice that the Mayo Clinic has not become extinct or closed its doors for lack of funds. When the public becomes educated to the fact that first-class service can only be obtained in Class A hospitals with good laboratories, then this cry will cease." While A. H. Sanford of the Mayo Clinic assures us that, "The public are appreciating more and more the value of laboratory work, and will soon ask to go to hospitals where they are not only charged for such service, but where they know they can get good laboratory work. I personally have seen a great change in the attitude of the public toward the Wassermann test alone. A few years ago I would conceal from the patient the true nature of the test. Now it is very common for a patient to say, 'Are you going to do a Wassermann test?' They are always satisfied when the reply is in the affirmative."

Thus hospitals may be reasonably assured that sentiment is crystallizing on all sides and it is only necessary to maintain a firm stand. All opposition will soon fade into thin air.

Patients have complained with reference to routine laboratory work, that it is unfair to charge for something which they have not requested. They look

to their attending physician for all service essential to their case. In many instances, those in attendance do not advise them with reference to the charge and in some cases have even told them not to pay it. "But," says M. L. Holm of Lansing Michigan, "all patients prefer to go to the better equipped hospitals. We know that patients are in the habit of paying for treatment only, but it is comparatively easy to train them to realize that medical service is not always measured by pills and stitches." As L. S. Lippencott of Vicksburg views it: "Education is the solution, and education must be applied to physicians as well as patients. The physician must be made to realize that laboratory service is necessary and must explain it to his patient. One factor that makes the physician slow in this regard is his unwillingness to admit to his patient that there is something which he cannot do. "Moreover," says F. C. Narr of Kansas City, "every hospital has a right, for the protection of its reputation, to require that every patient admitted to it be adequately studied, in order that a correct and complete diagnosis be made and proper treatment instituted. If the attending physician will not see to this, then the hospital, in self-protection, should." "The physician," thinks Robert F. Maul of Denver, "must be gradually taught that the laboratory is just as essential in the hospital as is the operating room."

Physicians, many of them, are open to censure for their apathy, not to say hostility, toward standardization; especially in the earlier days of the movement. Even fellows of the American College of Surgeons are not without fault in this direction. In the beginning there was almost a solid wall of opposition, characterized not only by indifference but many took the position that there was no precedent to justify the introduction of routine laboratory tests on hospital patients; that if this must be done, a technician might be employed on a small salary in order to obviate the added expense.

"It is usually the man of the old school who takes such a stand," says C. L. Klenk of St. Louis, "the more modern man, if he makes such a statement does so only because he is ignorant of the responsibility of the pathologist." "And," adds Phillip Hillkowitz, of Denver, "technicians cannot interpret the findings any more than we should expect nurses to perform operations." We are reminded by Sanford of the Mayo Clinic, that, "It takes about ten years for the profession to accept a new idea." "There is always opposition to anything that is out of the old order," states W. F. Thomson of Beaumont, Texas; while Ruth Gilbert of the New York Department of Health, at Albany, has to say: "If one compares the present attitude of physicians in regard to laboratory work with their attitude ten years ago, it will be noted that a marked change in favor of clinical laboratory service has occurred, and this tendency will undoubtedly continue, since medical schools are emphasizing more and more the importance of laboratory work. The unfortunate demand in certain localities for 'cheap' laboratory service seems one of the greatest hindrances to progress. Unless the character of the work is satisfactory, it is obviously better not undertaken. Nothing can be worse than false or misleading reports. The fact that an unsupervised technician does certain tests well, may lead physicians to place confidence in work less efficiently done; and thus

tend to discredit laboratory work in general in the minds of the physicians concerned.''

My personal opinion in this matter may be expressed as follows: The technician occupies the same relative position to the clinical pathologist that the nurse does to the surgeon. One would not expect the surgeon to cut sponges, clean instruments, prepare the operating room, make the patient ready for the operation, and clean up after it is over. Technicians, likewise, absorb much of the detail work in connection with the clinical laboratory. Many of them do become proficient in the technic of some of the lesser procedures, but in no case, are they qualified to correlate findings with the clinical condition of a patient, any more than a nurse in the operating room would be qualified to perform a laparotomy. True, many of them might conduct the mechanics of an operation very credibly but it would be unsafe, in the absence of the background of a medical education to permit them to assume the responsibility.

Our first thought, as we approach the question of financing clinical pathology in the modern hospital from the viewpoint of

The Clinical Pathologist, is the avoidance of any measure, the adoption of which might impede the development of clinical pathology as a specialty in medicine. It is to be noted that this specialty has evolved, not in connection with the hospitals, but in private laboratories in the medical communities. The development of the emolument of the clinical pathologist has been through the laboratory fee, which was commensurate with the amount of work involved and the refinement of the clinical diagnostic procedure. Pioneers in this branch have not been fettered by the heavy hand of servitude, but have, in common with their colleagues in other specialties, enjoyed the stimulus of adequate compensation for each transaction. These considerations, therefore, should guide those concerned with the growth of this phase of hospital standardization that due care be exercised to preserve, within the hospital, the same economic status for the clinical pathologist that prevails without.

As there exist various relatively common fees for different surgical procedures, which through custom, have become more or less stable; so likewise, has the force of precedent established a reasonable emolument for many of the operations of the clinical pathologist—as was shown by a recent survey among the pathologists of the country—which definitely established the value which time has placed upon the usefulness of the clinical pathologist. The question has been propounded on several occasions of late as to the amount of compensation which an experienced clinical pathologist should receive for full-time service.

Obviously it would be incompatible with good judgment to expect a surgeon of standing to relinquish his private practice and identify himself exclusively with one hospital at an income of, for example, less than ten thousand dollars per year. And there would be relatively few who would consider such a stipend equal to the sacrifice. As a matter of fact few well grounded surgeons would think of accepting a salaried position of this character. Not only because of inadequate reward, but of its servile limitations as well. To say nothing of the

inevitable precedent of paving the way to state medicine; few of us wishing to become institutionalized.

The clinical pathologist likewise, desiring to maintain his status as a consultant in medicine, will best cooperate with the hospitals in the establishment of adequate laboratory service, on a basis similar to that which obtains with relation to the surgical service or that of internal medicine.

The greatest difficulty in the financing of pathologic laboratories in connection with hospital standardization seems to have proceeded from the confusion arising from the two positions which clinical pathologists, as directors of hospital laboratories, have occupied. Primarily the clinical pathologist, especially in his private pursuits, is a consultant; comparable to other departments ine. In this capacity, his position is quite distinct from the director of tory maintained by a hospital for the maintenance of its standard, and its service made mandatory upon the physicians and surgeons enjoying its privileges. The financing of these two positions must necessarily develop in dissimilar ways. It is unnecessary, therefore, to refer to the methods or fees to be applied in consultation practice. The hospital will be compelled to sustain its laboratory and operatives as other additions and improvements are provided for. It would seem most reasonable as a basis for determination that the entire expense of the laboratory, the value of its space, the apparatus and material required, adequate compensation for director and technician, should be determined. This amount should be added to the gross income of the hospital and prorated in addition to the standard charge of accommodations which have prevailed. This implies the recognition of a dual capacity for the clinical pathologist who serves as a director or pathologist in the hospital laboratory. His compensation from the hospital is for his service as an indispensable part of modern hospital standard and is, therefore, essentially different from his employment as a consultant of choice of the attending physician or surgeon of the patient. In the latter instance he is obviously the choice of the physician and patient, while in the former he is the necessary adjunct of the hospital for the maintenance of its standard, and ofttimes an uninvited if not objectionable necessity to the attending physician; hence, the basis for criticism of implied injustice.

This has an analogue in the position occupied by the railroad surgeon or the physician retained by any other industry, for the maintenance of certain standards or ideals. Such men are secured at a stated retainer and their positions as consultants or private practitioners are in no way curtailed. The upward trend of humanitarian ideals requires the industries to establish certain standards of health protection for the promotion of which physicians or surgeons may be retained. Likewise, the advance of scientific medicine has made it necessary for the modern hospital to establish a department of clinical pathology which demands the services of a clinical pathologist.

When, however, the fulfillment of the minimum requirements of standardization have been reached, his position assumes that of a consultant and his remuneration should be determined accordingly.

Referring again to the minimum requirements of the College of Surgeons

which reads "That a clinical laboratory be *available* for the study of cases, etc.," "That a clinical pathologist be in charge and that certain tests be done upon the patients when they enter the hospital." It is apparent that the College does not require that all of the refinements of clinical pathology be furnished to the patrons of the hospital on the same basis as light and heat; but, that they be available; convenient; at hand; so that they may be utilized when required.

In conclusion it seems advisable to emphasize the dual position which has been occupied by the clinical pathologist associated with hospital service.

That the subject of financial support of clinical pathological pursuits essential to modern hospital standardization cannot be satisfactorily determined, without provision for each or both positions in which the clinical pathologist may be employed.

That portion of the service performed as a part of the hospital routine is an obligation of the hospital. Special pathological determinations which are deemed sufficiently necessary by the attending physician and surgeon in individual cases as to be required for the elucidation of the clinical state, or to determine the character and amount of treatment, is an obligation of the patient similar to an opinion rendered upon solicitation of the attending physician and compensation determined accordingly.

When such services assume the character of consultation contributing to the understanding and interpretation of the disease, the fee should be anticipated according to the standards established in private laboratories.

The security of the fee for such special service devolves upon the attending physician, or hospital, or both.

The freedom of consultation within the hospitals should not be restricted; but should be extended to all clinical pathologists of standing according to the preference of those concerned, as obtains in other specialties.

A MODIFICATION OF THE KOLMER REACTION*

By F. O. Huntsinger, Pharmacist, U. S. Navy, Great Lakes, Ill.

THE Kolmer technic in the complement-fixation test has been the subject of extensive research in the laboratory at the U. S. Naval Hospital, Great Lakes, Illinois, for the past eight months, and conclusions arrived at have pointed most favorably to a method which we now employ.

It would seem that in previous articles, appearing in different journals, that the great tendency is to cross check one test against another with the same sera; and meet with apparent failure, in noting an absence of hemolysis (or precipitation) in one test, and complete hemolysis in another, when the reverse should occur.

Many factors can account for this, such as erroneous technic and the presence of unknown bodies, passive or active in origin, which will or will not react

favorably to all tests. We believe the elimination of these differences without destruction of the luetic body is accomplished in the modified Kolmer reaction test used here.

In a series of tests in which the Noguchi and Kolmer technics were used simultaneously, we made a number of checks on cases which were positive for lues, malaria, tuberculosis (active), tonsillitis, Vincent's angina, and an undetermined febrile case. All were negative, except the luetic cases. When a large amount of serum is employed, inhibition of hemolysis will occur. In using a large amount of serum there is a chance that the fatty content, after the application of heat in the water-bath (56° C.), may have some bearing on the inhibition of hemolysis that occurs. For that reason small amounts of the serum are used unless a five tube test is used (as we frequently use when varying amounts of serum and antigen are employed). A negative serum has never given a positive reaction where the technic was correct.

The elimination of isolysins, or the rare autolysins, can be partially or completely accomplished by the washings of the unheated serum with the washed cells which are to be used in the test.

In the case of the Kolmer the patient's serum can be treated with washed sheep cells and thereby bind the native amboceptor that may be present. In the Noguchi, pooled washed human cells can be employed, exerting their action upon the agglutinins, precipitins and amboceptors, which may be present in the serum of patients who have received blood transfusions, et cetera, and have produced hemolysins (?) therein. The serum thus treated, having such bodies present which have any influence upon the cells to be employed, in the presence of human complement, will produce a haptophore group, provided the complement has not been destroyed. The native hemolysins being bound our test becomes more accurate.

A heterolysin being employed in the test in a known unit against a specific volume of a blood cell suspension, meets with a reduced number of obstacles.

After treating the serum thus, it is inactivated in the water-bath (56° C.) for twenty minutes. In our test, using only two tubes, we employ 0.2 c.c. of human serum in each tube of the Kolmer set up, and only 0.1 c.c. in the Noguchi. In the front tubes we employ 0.1 c.c. of acetone insoluble antigen (unit 0.1 c.c.).

The guinea pig complement is made up in the dilution of one part guinea pig serum to nineteen parts of sterile physiologic salt solution. The unit is determined by titration, with double the amboceptor unit and one mil of the 2 per cent suspension of sheep cells in sterile physiologic salt solution. The complement unit is doubled in the test. Each tube in the test then receives this doubled unit of complement. Shake well and incubate for one hour at 37.5° C. At the end of the hour, we remove the rack containing the tests from the incubator and immediately add the antisheep amboceptor (unit doubled) and 1 c.c. of a 2 per cent suspension of sheep cells. Shake well and incubate for one hour at 37.5° C., shaking the tubes well every fifteen minutes for the first forty-five minutes.

Control tubes consist of the positive 4-plus serum, positive 4-plus spinal fluid, negative serum, negative spinal fluid, antigen control on each antigen

employed, amboceptor control, guinea pig complement control and sheep cell control.

The spinal fluids are not inactivated; five tubes are run with a control on each. Amounts of spinal fluid employed in the tubes are as follows: 0.2 c.c., 0.4 c.c., 0.6 c.c., 0.8 c.c., and 1.0 c.c.

Titrations for the unit amounts to be used in the Noguchi test are the same forms of procedure, except that we employ one-half c.c. of a .2 per cent suspension of human cells. Incubation is for only one-half hour. We then add the antihuman amboceptor (double unit) and one-half c.c. of a 2 per cent suspension of human cells. Shake well and incubate for one-half hour at 37.5° C.

We have used both the Noguchi and the Kolmer tests as checks against one another for a long period of time, but lately have run only the Kolmer.

I wish to thank Commander O. J. Mink (MC), U. S. Navy, for his reading and correcting the original manuscript.

CONCLUSION

1. That the Kolmer is the most reliable of all the complement-fixation tests in the detection of lues.

2. That native hemolysins can be destroyed or grouped by proper treating of the fresh blood serum.

3. That malaria in the Kolmer reaction will not give a positive four-plus reaction.

4. That febrile conditions other than lues will not give a positive Kolmer (Yaws influences this test in the tropics).

5. And that the incubator (37.5° C.) or the water-bath method is preferred to the ice-box fixation method.

REPORT OF AN UNUSUAL CASE OF CONGENITAL CARDIAC DEFECT (COR TRILOCULARE) ASSOCIATED WITH TRANSPOSITION OF PULMONARY ARTERY AND ARCH OF AORTA*

BY M. G. WOHL, M.D., OMAHA, NEBRASKA

CASES of cardiac congenital defects in man have been the subject of classic discussion by writers such as: Rokitansky,[1] Lebert-Schroter,[2] Keith,[3] Abbott,[4] Schwalbe.[5] For detailed critical analysis of such cases the reader is referred to the authors mentioned.

The case to be described below is quite unique because of the multiplicity of the cardiac defects, accompanied by transposition of pulmonary artery and thoracic aorta.

Clinical Notes.—Female, white, seven months old, of American parentage in whom no history of syphilis, tuberculosis, or any other chronic disease was elicited. The child was full term and normal delivery. Weight at birth six and one-half pounds. She breathed

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almost immediately on delivery, yet she was noticed to be blue at the time of delivery. The cyanosis kept on increasing with the age of the child and remained until her death.

Past History.—Baby has been blue since birth; her cyanosis getting worse when she cried; she did not thrive. Dr. Newell Jones, Omaha, was called in to regulate her feedings. At the time the doctor saw her, she was three and one-half months old; she was cyanotic, poorly nourished, and had slight clubbing of the fingers. There was a palpable thrill over the precordium and systolic murmur was heard over lower sternum which later became audible all over precordium. Pulse rate varied from 130 to 170 per minute. Her temperature was elevated a degree and a half; a few days prior to her death, her temperature became subnormal.

Autopsy Findings.—The body is that of a female, white infant, seven months old, 58 centimeters in length. There is slight edema of legs and feet. Pronounced cyanosis of lips, face, hands and feet. Marked clubbing of fingers. Thymus normal size. The pleural cavity contains light amber-colored fluid. Lungs moderately congested. No other pathologic findings noticed.
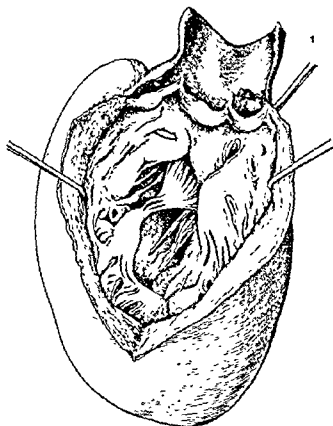
Fig. 1.—Interior of right ventricle. Note thickness of right ventricular wall. Aorta bears three semilunar cusps. *1*, A probe passes through tricuspid orifice from the auricle.

Abdomen: Contains free serous fluid. Normal spleen absent. In the site normally occupied by that organ, there are four accessory splenules; one the size of an olive, the other three the size of a small pea. This is unusual; splenules accessory to normal spleen being not uncommon, however, accessory splenules without the spleen proper is rather rare.

Liver: Weight 190 grams, moderately congested.

Gastrointestinal tract, apparently normal. Gall bladder and pancreas normal; kidneys show cloudy degeneration. Adrenals apparently normal. Sex organs normal. Abdominal aorta normal.

Heart: Pericardium free from adhesions; contains 15 c.c. of clear yellowish fluid. The heart itself is enlarged, weighing 82 grams, and measuring diagonally 9 by 7½ cm. The enlargement is due to hypertrophy of right ventricle and dilated auricles.

The most striking anomaly seen upon the anterior aspect of the heart is the origin of the aorta, which arises from the right ventricle, at the usual site of origin of the pulmonary artery.

The pulmonary artery arises from the left ventricle at the normal place of origin of the aorta. It does not communicate with the ventricular cavity proper, as it is atresic in its lowest 2 cm.; its lumen ending blindly at site of atresia. The inferior vena cava enters the auricle from below at normal site. The superior vena cava, however, opens abnormally wholly into left ventricle. The aorta is 1.5 cm. in diameter at its base and gives off two main coronary branches. The left coronary is seen anterior to the pulmonary artery, passing posteriorly to auricles where it divides into an auricular and anterior circumflex branch. The ductus arteriosus is patent throughout, measuring 1 by 0.5 cm. and connects pulmonary artery with aorta, just proximal to primary division of the latter.

Fig. 1 shows interior of right ventricle. The right ventricle is hypertrophied and dilated; its wall being 2 cm. thick and forming the whole cardiac apex. The aorta, bearing three semilunar cusps of equal size but
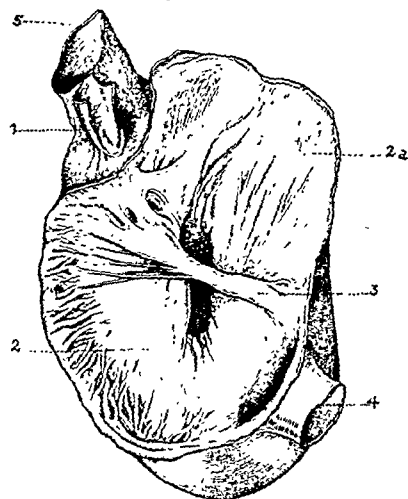
Fig. 2.—Interior of auricular portion of heart. A coronal section has been removed, exposing the interior of both auricles from above.
1, Atresic pulmonary artery; 2, 2-a, interior of auricles bisected by muscular band; 3, defective auricular septum; 4, inferior vena cava, opening into right auricle from below; 5, superior vena cava, opening into left auricle.

with thickened contracted borders, rises from the conus of the right ventricle in the position normally occupied by the pulmonary artery, as noted above. The conus is short and its muscular pillars unusually irregular and hypertrophied. A large tricuspid valve having well formed infundibular septae is seen. Its chordae tendinae are fused and thickened. The tricuspid orifice is dilated, giving rise to an insufficiency. The left ventricular cavity is markedly diminished as compared with the right, its wall is also thin, measuring 0.2 cm. The pulmonary artery, already described as arising from normal atresic site, has a thin wall, and is of diminutive size ending below in a glistening culdesac.

The common auricular cavity is partially divided by a narrow thickened muscular band that traverses it obliquely above the center, anteroposteriorly at about normal lines of attachment. This band is strongly attached by its extremities to the musculature of the auricles; it sends on the right side several additional chords, tendinous in nature, to be inserted in a fanshaped fashion on

posterior auricular wall. Aside from this band there is no other septal tissue. The auricles communicate widely, therefore, with each other above and below the band. The band represents apparently an incomplete septum primum. The inferior vena cava enters the right auricle from behind and below to the right of the defective septum. The two auricular lumina, therefore, form a common cavity in wide communication above and below this band. The interventricular septum is complete, separating the right ventricle wholly from the left. The left ventricle which is not shown in the drawing presents walls strikingly thin and weak in comparison with the right. The heart has, therefore, two separate ventricular cavities each opening into a common auricular lumen.*

ANATOMIC DIAGNOSIS

Complete reversal of origin of aorta and of pulmonary artery that arise from normal sites of each other. Aortic insufficiency resulting from the fact that the small contracted aortic valves do not meet, as described above; aplasia and diminution of left ventricle with atresia of mitral orifice; hypertrophy and dilatation of right ventricle. Auricular septum markedly defective above and below. Apparently persistent and much enlarged ostium primum and secundum. Tricuspid orifice dilated. Accessory splenules. Passive congestion of liver. Cloudy degeneration of kidneys.

COMMENT

We are dealing with a rare cardiac anomaly. There are only ten cases of defects of upper part of septum described in the literature.[6]

From a critical survey of the cases reported, it would seem that defects in the interauricular septum are commonly associated with hypoplasia of the aorta and dilatation of the pulmonary artery. Our case is unique in that the aorta and pulmonary artery were transposed; the aorta was dilated and pulmonary artery stenosed. The pulmonary stenosis would have caused immediate death of the child were it not for the presence of the open ductus arteriosus. This, doubtless, permitted a retrograde flow of blood from aortic arch to pulmonary arterial system which was normal above the site of atresia already noted; the blood, therefore, reached the pulmonary arterial system in an unusual manner. The mechanism in production of this auricular defect may be best understood through the application of the principles of the normal embryologic development as first pointed out by Rokitansky.[7]

"The auricular septum is known to develop in two parallel planes (Born, His); which grows downward from the roof of the common auricle at a very early stage. Of these two, that developing first, the septum primum, lies on the left side; as it grows downward, an opening remains for some time at its lower border above the portion of the interventricular septum formed by the endocardiac cushions. This is known as the ostium primum. Gradually, as the primary septum grows downward, this ostium primum closes in, and a second opening appears above, in the upper part of the primary septum. This is the ostium secundum or foramen ovale which is guarded by the septum secundum.

*It is to be regretted that during preparation of the drawings the specimen became mislaid and other detailed study of it became impossible.

The latter arising from the right auricle to the right of the septum primum, arches downward, acting as a valve in the embryo and forming annulus ovalis of postnatal life.''

The connection of the right and left auricular lumina below and above the auricular band described above is apparently a partially persistent ostium primum. The opening below the band is due to failure of this septum to grow down and fuse with the septum below. This assumption is based upon the fact that normally the closing of the ostium primum, by a downward growth of the septum primum, effects a permanent separation of the auricles at this site. The absence of auricular septum above this band is probably the result of an abnormally large ostium secundum or foramen ovale, in the formation of which, the whole upper part of the septum as well apparently as the septum secundum have disappeared. The transposition of the vessels can be accounted for by deviation of aortic septum within the truncus aorticus and by faulty union with the ventricular cavities. That pulmonary stenosis is due to congenital defect rather than to intrauterine endocarditis seems probable, on account of the presence of the other cardiac defects.

I wish to express my appreciation to Dr. Maude E. Abbott and Dr. James McDonald for valuable suggestions made during the preparation of the paper.

REFERENCES

[1]Rokitansky: Defecte Der Scheidewande des Herzens. Vienna, 1875.
[2]Lebert-Schroter: Handbuch der speziellen Pathologie und Therapie, Leipzig, 1879, vi.
[3]Keith: Hunterian Lectures on Malformations of Heart, Lancet, 1909, ii, 359-433.
[4]Abbott, M. E., Osler, and McCrae: Mod. Med.
[5]Schwalbe: Morphologie der Misbildungen des Herzens, Jena, 1910, p. III, Lieferung III
      p. 388.
[6]Abbott, M. E., and Kaufmann, Joseph: Jour. Path. and Bacteriol., 1910, xiv.
[7]Rokitansky, quoted by M. E. Abbott: Jour. Path. and Bacteriol., 1910, xiv, 532. Quotation slightly modified by writer.

PRODUCTION OF IMMUNITY WITH A SOLUBLE ANTIGEN OF THE PNEUMOCOCCUS*

By N. S. Ferry, Ph.B., M.D., and L. W. Fisher, B.S., Detroit, Mich.

DURING the past two years the writers have been carrying on some extensive experimental work for the purpose of determining the immunizing properties of bacterial antigens prepared after various methods; and as a result of this work have reported some unusual and unexpected findings.[1]

Having tested over three hundred antigenic preparations from several different organisms during this time, we have been compelled to modify in many respects our previous conceptions of bacterial antigens, especially as to their origin.

While testing broth cultures of some of the so-called endotoxin producing organisms, as the typhoid bacillus, colon bacillus, pneumococcus and others, it was found that the antigenic properties of the filtrates were of extremely high value, providing they were obtained from growths incubated at the right temperature and for the proper length of time. The optimum temperature proved to be about 37° C. while the incubation time, which gave the best results, was between eighteen and twenty-four hours.

In an endeavor to carry out these experiments as qualitatively as possible, twenty-four broth cultures were divided into three parts or fractions; one to be tested as such, one to be centrifugalized for the preparation of the filtrate fraction, termed the "centrifugate" and one to be centrifugalized for the sediment (diluted to original volume with salt solution). These three fractions were sterilized by the addition of 0.3 per cent cresols. Their antigenic properties were determined by injecting them intravenously into rabbits in three or four doses and testing the serums, according to various serologic methods, for antibody content. The results showed that the filtrates in many instances equalled and, in some cases, were superior in antigenic value to both the original broth culture and the sedimented bacteria. In practically all tests the sedimented bacteria were found to be inferior in antigenic properties to the whole culture and in many instances also to the filtrate. (See Table I.)

It was found that if broth cultures were incubated over twenty-four hours, the antigenic properties diminished proportionately, showing that there must be something formed in the media or secreted by the organisms antagonistic to the antigens. (See Table II.)

These results have demonstrated quite conclusively that the antigens of these organisms are found in the filtrates of broth cultures in a high degree of availability and have also convinced us that the antigens are not entirely endotoxic in nature. They must be more or less loosely bound to the cells.

From these findings, it was concluded that if the antigens were loosely bound to the cells or were a component of the ectoplasm, they would be more apt to be found in greater concentration in an aqueous solution obtained by merely washing the organisms grown on solid media than if an attempt were made to extract them by chemical or physical means.

In an attempt to prove this point washings were prepared as follows: The organisms were grown on solid media for twenty-four hours, taken off in salt solution, shaken in mechanical shaker for a few minutes and then immediately

TABLE I

TEST OF THE ANTIGENIC VALUE OF BROTH CULTURES OF VARIOUS ORGANISMS CONTAINING 1000 MILLION ORGANISMS PER C.C.

ANTIGENS	TITER OF IMMUNE SERA		
METHOD OR PREPARATION	AGGLUTIN-ATION	COMPL. FIX.	BACTERIO-TROPIN
Broth culture of B. typhosus (1000 million organisms per c.c.)	1:18000	1:10000	1:2800
Centrifugate from broth culture	1:16000	1:10000	1:6000
Sediment from broth culture diluted to original volume	1:4000	1:700	1:450
Broth culture of B. coli	1:18000	1:150	
Centrifugate from broth culture	1:12000	1:600	
Sediment from broth culture diluted to original volume	1:900	1:200	
Broth culture of pneumococcus Type I	1:80	0	
Centrifugate from broth culture	1:70	0	
Sediment from broth culture diluted to original volume	0	0	
Broth culture of streptococcus	1:500	1:10	
Centrifugate from broth culture	1:600	1:10	
Agar suspension (1000 million organisms per c.c.)	1:800	1:60	
Agar washings	1:800	1:50	
Broth culture of gonococcus	1:400	1:10	
Centrifugate from broth culture	1:400	1:10	
Agar suspension of equal number of organisms	1:500	1:100	
Agar washings	1:600	1:100	

TABLE II

TEST OF THE INFLUENCE OF THE INCUBATION TIME, PEPTONE AND SALT UPON BROTH CULTURES OF B. COLI

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Plain Broth		
Incubated 5 hours (centrifugate)	1:150	1:10
" 24 hours "	1:8000	1:600
" 5 days "	1:300	1:50
" 10 days "	1:300	1:50
Plain broth without peptone		
Incubated 5 hours (centrifugate)	1:60	1:40
" 24 hours "	1:150	1:40
" 5 days "	1:60	1:10
" 10 days "	1:250	1:10
Plain broth without peptone and salt		
Incubated 5 hours (centrifugate)	1:40	1:15
" 24 hours "	1:40	1:10
" 5 days "	1:200	1:10
" 10 days "	1:80	1:15

passed through a Sharples centrifuge. The entire operation did not consume over half an hour from the time the growth was taken from the incubator to the final stage in the process.

These washings have been tested together with antigens prepared by other well-known methods and have been found to be extremely high in antigenic value. When injected into rabbits, following the method described above for testing broth cultures, the washings were found capable of producing antisera of very high titer. (See Tables III and IV.)

The washings have also been used with success in the rôle of antigens in the complement-fixation test. (See Table V.)

Recently[2] with these same washings prepared from the Streptococcus hemolyticus scarlatinae we have been able in collaboration with Dr. Pryer to produce a positive skin reaction in individuals susceptible to scarlet fever (Dick reaction), which reaction would become negative if the washings were

TABLE III

TEST OF THE ANTIGENIC VALUE OF VARIOUS ANTIGENS PREPARED FROM THE TYPHOID, PARA. A AND PARA. B, BACILLI DILUTED SO THAT ALL CONTAINED 1000 MILLION ORGANISMS PER C.C.

ANTIGENS		TITER OF IMMUNE SERA	
METHOD OF PREPARATION		AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Typhoid vaccine		1:9000	1:1000
Typhoid-paratyphoid vaccine comb.	B. typhosus	1:6000	1:100
	B. para. A.	1:5000	1:50
	B. para. B.	1:8000	1:250
Typhoid-paratyphoid washings	B. typhosus	1:16000	1:750
	B. para. A.	1:2000	1:750
	B. para. B.	1:20000	1:750
Typhoid-paratyphoid broth centrifugate	B. typhosus	1:10000	1:175
	B. para. A.	1:3000	1:60
	B. para. B.	1:12000	1:750
Typhoid glycerol vaccine	B. typhosus	1:6000	1:90
Typhoid serobacterin	B. typhosus	1:5000	1:75
Detoxicated T.A.B. vaccine	B. typhosus	1:2000	1:25
	B. para. A.	1:20	1:5
	B. para. B.	1:200	1:35
Residual T.A.B. vaccine	B. typhosus	1:2000	1:25
	B. para. A.	1:20	1:5
	B. para. B.	1:200	1:35

TABLE IV

TEST OF THE ANTIGENIC VALUE OF VARIOUS GONOCOCCUS ANTIGENS DILUTED SO THAT ALL CONTAINED THE SAME NUMBER OF ORGANISMS

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Agar washings of gonococcus	1:2000	1:100
Broth centrifugate of gonococcus	1:400	1:50
Gonorrhea (comb.) vaccine	1:900	1:90
Residual gonococcus vaccine	1:80	1:10
Gonococcus glycerol vaccine	1:100	1:70
Gonococcus serobacterin	1:30	1:50

TABLE V

TEST OF VARIOUS TYPHOID ANTIGENS WHEN USED AS SUCH IN THE COMPLEMENT-FIXATION
TEST AGAINST A KNOWN ANTITYPHOID SERUM

ANTIGENS	IMMUNE SERA
METHOD OF PREPARATION	HIGHEST DILUTION FIXING COMPLEMENT
Mixture of broth centrifugate and agar washings of B. typhosus	1:90
Agar washings of B. typhosus	1:80
Agar washings of typhoid-paratyphoid mixture	1:60
Broth centrifugate of typhoid-paratyphoid mixture	1:90
Broth centrifugate of B. typhosus	1:70
Autolysates of B. typhosus	0

TABLE VI

TEST TO DETERMINE THE INFLUENCE OF REPEATED WASHING UPON THE ANTIGENIC VALUE
OF SUSPENSIONS MADE FROM THE WASHED ORGANISMS. B. TYPHOSUS WAS USED AS THE
TEST ORGANISM. 1000 MILLION ORGANISMS PER C.C.

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTINATION	COMPLEMENT FIXATION
Agar suspension	1:8000	1:325
Agar washing	1:11000	1:500
Washed organisms	1:4000	1:600
Broth growth	1:7000	1:300
Broth centrifugate	1:8000	1:250
Broth sediment	1:35000	1:600
Broth sediment washed	1:3000	1:C00
Washings from antigen No. 63	1:1500	1:50

mixed with convalescent scarlet fever serum, similar to the results obtained by the Dicks of Chicago,[3] Zingher of New York,[4] Branch and Edwards,[5] Gatewood[6] and others with the Dick toxin.

Returning to the sedimented bacteria from which the washings were obtained, it was found, as with the sediment from the broth cultures in the first experiment, that the washed organisms were relatively low in antigenic properties. These sedimented bacteria washed the second time have yielded antigens even lower in antigenic properties than the sediment after the first washing and the second washings from this sediment have also suffered more or less in antigenic properties. (See Table VI.)

In carrying this work further, autolysates were prepared from the organisms, both before and after being washed, and it was found that they were even lower in antigenic value than the second washings from the unautolyzed sediment. This was especially marked of the autolysates from the washed organisms, showing that washings did not depend upon the autolyzation of the organisms for their antigenic properties. (See Table VII.)

Another experiment, which proves conclusively the antigenic value of these washings and centrifugates and would also tend to lend some weight to the suggestion that antigens are nonprotein in nature, was carried out by precipitating several different washings and broth centrifugates with various substances until a protein-free reaction was produced. These apparently protein-

TABLE VII

Test to Determine the Influence of Washing Organisms, Both With and Without Long Shaking, upon the Antigenic Value of the Autolysates of the Sedimented Bacteria. Suspension of B. Typhosus Was Used, 1000 Million Bacteria per c.c.

ANTIGENS	TITER OF ANTISERA	
METHOD OF PREPARATION	AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Agar washings	1:12000	1:750
Broth centrifugate	1:12000	1:375
Autolysates from residue of agar washings	1:3000	1:100
Autolysates from residue of broth centrifugate	1:7000	1:500
Agar washings after shaking suspension 24 hr.	1:5000	1:500
Autolysates from residue of previous antigen	1:2000	1:100
Autolysates from agar growth not previously extracted by shaking	1:7000	1:150

free antigens were injected into rabbits, according to the usual method, and the serums tested for the presence of antibodies with positive results in several cases. (See Table VIII.)

From these experiments, it was suggested that the antigens of the organisms studied were more liable to be ectoplasmic than endoplasmic in origin, and for that reason this type of an antigen has been termed an "ectoantigen."

The more recent work which we wish to report at this time includes experiments carried on with washings or ectoantigens prepared from Type I pneumo-

TABLE VIII

Test of the Antigenic Value of Antigens Apparently Protein Free

ANTIGENS			TITER OF IMMUNE SERA	
ORGANISMS	METHOD OF PREPARATION	METHOD OF PRECIPITA-TION	AGGLUTINA-TION	COMPLE-MENT FIX-ATION
B. typhosus	Washings	Phosphotungstic acid	1:200	1:25
B. typhosus	Broth centrifuge	Uranium acetate	1:80	1:25
B. coli	Washings	Acetone	1:100	1:5
B. coli	Broth centrifuge	Uranium acetate	1:200	1:50
B. coli	Broth centrifuge	Phosphotungstic acid	1:2000	1:75
Pneumococcus	Phenolized centrifugate	Acetone	1:40	1:10
Pneumococcus	Agar washings	Acetone	1:80	1:25
Pneumococcus	Phenolized centrifugate	Phosphotungstic acid	1:40	1:10
Pneumococcus	Phenolized centrifugate	Uranium acetate	1:40	1:10
Streptococcus	Agar washings	Acetone	1:800	1:25
Streptococcus	Phenolized centrifugate	Acetone	1:400	1:5
Gonococcus	Agar washings	Acetone	1:100	1:10

coccus. The organism used for all the experiments was a strain obtained from the Hygienic Laboratory, Washington, for the preparation of Type I antipneumococcic serum. It has repeatedly been tested, and according to all known serologic tests, has proved to be true to type with a minimum fatal dose to white mice of from one hundred millionth to one billionth cubic centimeter of a twenty-four hour broth culture.

In preparing the washings the seed cultures were grown on blood agar and transplanted to large flasks of ascites agar. These were incubated twenty-four hours and washed off with salt solution and divided into five lots.

TABLE IX

TEST TO DETERMINE THE VALUE OF TYPE I PNEUMOCOCCUS WASHINGS BY MEANS OF ACTIVE PROTECTION TESTS ON WHITE MICE

LOT	MOUSE	DILUTION OF CULTURE	RESULTS
I	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
II	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
III	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
IV	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	"
	5	0.001	Died
	6	0.01	"
	7	0.1	"
	8	1.	"
V	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	"
	5	0.001	"
	6	0.01	Died
	7	0.1	"
	8	1.	"

M. F. D. of culture—0.000000001 c.c.

Lot I. The suspension was subjected to a temperature of 60° C. for one hour, after which it was treated by the usual method for the preparation of washings.

Lot II. This was washed first and then the washings were subjected to a temperature of 50° C. for one hour.

Lot III. This was treated as Lot II but heated only to 40° C. for one hour.

Lot IV. This suspension was treated with 0.3 per cent cresols and allowed to stand twenty-four hours before treatment as a washing.

Lot V. This was treated at once without heat or cresols, according to the previously described method of preparing washings.

To all of the final products except Lot IV, 0.3 per cent cresols were added as a preservative. White mice were given two doses of 0.5 c.c. of these extracts

subcutaneously one week apart and one week after the last dose were injected intraperitoneally with varying doses of the live organisms. (See Table IX.)

Results.—The results showed that Lot I which had been heated to 60° C. for one hour before being washed protected against 10,000 M. F. D. and the same was true of Lots II and III which had been heated to 50° C. and 40° C., respectively. Lot IV which had been sterilized by the addition of 0.3 per cent cresols for twenty-four hours before washing, protected against 100,000 M. F. D. while Lot V extracted a few minutes with salt solution, protected against at least 1,000,000 M. F. D.

Discussion.—From these results it is evident that an aqueous extract of Type I pneumococcus may be obtained from the washings of the organisms, fairly stable at a temperature of 60° C. for one hour with antigenic properties extremely high, without resorting to chemical extraction; and suggests the possibility of the use of such an extract for prophylactic purposes against pneumonia in the human.

REFERENCES

[1]Ferry and Fisher: Brit. Jour. Exper. Path., 1924, v, 185 and 205.
[2]Ferry, Pryer and Fisher: To be published.
[3]Dick and Dick: Jour. Am. Med. Assn., 1924, lxxxii, 265.
[4]Zingher: Ibid., 1924, lxxxiii, 432.
[5]Branch and Edwards: Ibid., 1924, lxxxii, 1260.
[6]Gatewood: Ibid., 1924, lxxxiii, 494.

COMPLEMENT FIXATION IN TUBERCULOSIS WITH PARTICULAR REFERENCE TO A GROUP OF 102 POSITIVE REACTIONS*

By Lloyd R. Jones, U. S. Veterans' Hospital No. 98, Castle Point, New York

DURING the past few years it has been the purpose of this laboratory to study the complement-fixation reaction as applied to the blood sera of patients, in the diagnosis of tuberculous disease. It is recognized that the test has peculiar limitations and the purpose of this study has been to determine the specificity of a positive reaction. The problem has been attacked from the viewpoint of determining the value of the positive reaction as obtained from the blood serum.

With one particular lot of antigen, which after extensive trial, with many tuberculous and clinically nontuberculous sera, was found to possess desirable antigenic properties, the blood sera of 617 patients were subjected to the test. Of the number tested, 102 gave positive reactions of either "+++" or "++++" degree (basis: four plus representing complete inhibition of hemolysis).

Of the more than 400 patients giving a negative reaction to the test, it was recognized that many of the number were tuberculous. However, the purpose

of the present discussion is merely to determine the incidence of tuberculous disease among those individuals giving a positive reaction.

The technic of the reaction as made in this particular study was as follows:

a. *Antigen.*—Necessarily an essential element in making a satisfactory test is a reliable antigen of established antigenic properties. Several strains of B. tuberculosis were grown in flasks of nutrient glycerin bouillon in large volume, then removed and washed with saline solution, centrifuged and later removed to a drying chamber. Masses of the partially dried bacilli were then transferred to a mortar and ground with a pestle for more than three hours, only slight amounts of distilled water being added. Smears made of the material and stained with fuchsin showed considerable disintegration of many bacillary bodies. After the grinding process, distilled water was added in amount sufficient to make the resulting mixture markedly opaque. One half of one per cent of phenol was added as a preservative and the mixture heated in a water-bath to 70° C. for twenty minutes. For use, one to ten dilutions were made in physiologic saline solution and standardization effected by titration with known positive and negative sera. The dose of antigen for use in the test was considered as an amount two times the least amount giving complete inhibition with a positive serum, in the event that twice that tentative amount did not inhibit hemolysis with a negative serum. Lots of antigen showing excessive anticomplementary or irregular antigenic properties were discarded.

b. *Patient's Serum.*—Clear blood serum was regularly inactivated at 56° C. for thirty minutes on the day following withdrawal.

c. *Complement.*—Pooled blood sera from two or more guinea pigs. The serum from each pig having been previously tested for fixability with this antigenic system. For use, one to ten dilutions were made in physiologic saline solution.

d. *Hemolytic Amboceptor.*—Blood serum of a rabbit highly immunized to sheep erythrocytes.

e. *Sheep Cells.*—Obtained from the sheep and used as a 5 per cent suspension of the washed cells.

The tests were performed in a manner closely paralleling the classical Wassermann test. Two units of complement, the dose of antigen and 0.2 c.c. of patient's serum were incubated for one hour in the water-bath at 37° C., whereupon two units of hemolytic amboceptor and 0.5 c.c. of the cell suspension were added to each tube and readings made after a thirty minute period of secondary incubation. Concerning the length of the primary incubation period, it was noted that a greater degree of specific fixation was obtained with an incubation period of one hour at 37° C. than was obtained at "ice box" temperature (6° C.) for four hours, a distinct variation from the experience frequently encountered in working with bacillary antigens.

An attempt was made to classify the various physical findings on the 102 patients giving a "+++" or "++++" reaction to the test. Competent examiners will frequently disagree on a diagnosis of clinical tuberculosis; hence, the clinical diagnoses as listed cannot be considered as an absolute index to the accuracy of the test. Data considered in basing a diagnosis of clinical tuberculosis were obtained from the history, temperature and weight charts, physical examinations, sputum examinations, Roentgen plates and interpreted in manner as is customary among most authorities on tuberculosis.

Pulmonary tuberculosis, active, with B. tuberculosis present in the sputum.   Cases 55

Pulmonary tuberculosis, active clinically, well defined cases but with negative sputum.   Cases 22

Pulmonary tuberculosis quiescent clinically, well defined evidence of former tuberculous process   Cases 21

Pulmonary tuberculosis negative clinically. Syphilitic. Wassermann positive.   Cases   1

Pulmonary tuberculosis negative clinically.
Diagnosis: Laryngitis   Cases   1

Pulmonary tuberculosis negative clinically.
Diagnosis: Asthma   Cases   1

Pulmonary tuberculosis negative. Otherwise negative clinically   Cases   1

The results here given, though encouraging, are not of sufficient scope to prognosticate the value of the reaction. In surveying these cases the purpose has been to determine, in so far as is clinically possible, the incidence of definite tuberculous disease among individuals giving a positive reaction to the test.

Concerning the so-called "cross-fixation" or nonspecific fixation with luetic sera, frequently reported, it was noted in this investigation that ten of the individuals gave a positive Wassermann reaction concomitantly; however, in each case diagnoses of dual disease: viz., syphilis and tuberculosis were made in all except one, who was considered as being clinically tuberculosis-free. Of the ten cases referred to, five were available for specific therapy and after intensive antiluetic treatment, with the disappearance of Wassermann reaction, it was noted in all cases that positive reactions with the complement-fixation test for tuberculosis persisted. So it is thought that with the use of a properly prepared antigen and properly controlled technic, nonspecific fixations with luetic sera need not be frequently encountered.

In making the test with the blood sera of patients who were in a far advanced stage of tuberculosis, it was noted that negative reactions were obtained in approximately 50 per cent of such cases. Presuming that we are dealing with a true antibody reaction, this anomaly is readily explained by the supposition that enormous amounts of tubercle antigen, are elaborated into the blood stream, incident to the extensive tuberculous process in a terminal case. By absorption, neutralization or other physiochemical processes the "reaction-bodies" necessary for the fixation of complement are rendered inert, hence, when such a condition is established, negative complement-fixation reactions will follow.

SUMMARY

1. An antigen preparation for the test should be accepted only after thorough preliminary trial demonstrating satisfactory antigenic qualities. The amount to be used in the test can be determined only after careful experimenta-

tion and must be such as permits a very fine quantitative adjustment between complement, antigen and patient's serum.

2. Several control sera should be regularly employed to detect evidence of false fixation, antigen deterioration, etc.

3. Limited observations, herein reported, would tend to promote the belief that positive reactions as obtained with the test when correctly performed, indicate with a high degree of accuracy the presence of tuberculosis "reaction-bodies" irrespective of whether the disease is then clinically active or quiescent.

THE INFLUENCE OF EXPERIMENTAL ATHEROSCLEROSIS UPON THE SYSTOLIC BLOOD PRESSURE IN RABBITS*

BY SHEPARD SHAPIRO, M.D., AND DAVID P. SEECOF, M.D., NEW YORK CITY, N. Y.

INCREASES in systolic blood pressure have been reported in rabbits following the prolonged feeding of cholesterol in oil, or of cholesterol containing substances administered for the purpose of producing atherosclerosis, by Fahr,[1] Van Leersum,[2] Schmidtmann[3] and Schönheimer.[4]

Three different methods for measuring the blood pressure were used by these authors. The method employed by Fahr[1] is as follows: A rubber cuff connected to a manometer is placed around the abdomen of the rabbit; air is forced into this cuff until the abdominal aorta is occluded, as indicated by the disappearance of the femoral pulse. The pressure in the cuff is then gradually released, and the instant the first femoral pulse returns the reading is taken and recorded.

In seven of the ten rabbits to which he had fed egg yolk and milk and which showed atherosclerosis at necropsy, Fahr observed increases in the blood pressure. In two of four rabbits which he had suspended, head down, daily, he observed similar elevations in blood pressure. The arteries in these latter four rabbits showed no atherosclerosis. Fahr observed cardiac hypertrophy in those rabbits in which he found hypertension following egg yolk feeding; and claims, further, that there was in these animals a parallelism between the degree of atherosclerosis, the cardiac hypertrophy, and the hypertension.

There is an obvious objection to Fahr's method. Constriction of the rabbit's abdomen is unphysiologic and per se gives rise to uncontrollable alterations in blood pressure.

Van Leersum[5] used a method which he published in 1911, and which briefly is as follows:

The carotid artery is isolated and a rubber tube passed around it. The wound is then permitted to heal with the rubber tube in position. At the time the reading is taken (from two to four weeks after the operation) a rubber cuff is joined to the tube and held in place by forceps. This cuff is connected to a

rubber bulb and a manometer, and air is forced into the tube until the artery is occluded. The pressure is then gradually released and at the instant the first pulsation is felt by palpation of the vessel the reading is made. This is recorded as systolic pressure. By this means Van Leersum[2] observed an increase in systolic blood pressure of 30 per cent after feeding powdered liver to rabbits for long periods of time.

This method offers a very direct means for determining blood pressure and would be an excellent one, provided the artery could be thus isolated without altering the compressibility of its walls through inflammatory reactions. This objection seems to render the method both impractical and prohibitive.

Van Eweyk and Schmidtmann,[6] therefore, in 1922, published the following method:

The rabbit is fastened to a board. The circulation in one of the forelegs is occluded by means of a tourniquet. A rubber cuff, which is connected to a bulb and manometer, is then placed around the upper portion of the leg. Air is forced into the cuff until the manometer reads 160 mm. mercury. The tourniquet is then removed. A small incision through the skin is made below the point of occlusion and the pressure in the cuff is gradually reduced. When the blood appears in the small arteries of the exposed fascia, the manometer is read and this reading recorded. The incision is sewed up and iodine applied. Using this method, Schmidtmann[2] observed a rise in blood pressure following the feeding of powdered liver in six rabbits, confirming in this one respect Van Leersum. He reports, further, that the blood pressure fell after powdered liver was discontinued and rose again when feeding with liver was resumed.

At necropsy, Schmidtmann found intimal changes present in the aortas of these rabbits. It should be emphasized that Van Leersum, in the eight rabbits in which he observed elevations in blood pressure (as determined by his method) following feeding with powdered liver, failed in all cases to find atherosclerosis on postmortem examination. Schmidtmann also claims that there is a direct relationship between the severity of the vascular lesion and the height of the blood pressure; and concludes that the cholesterol contained in the powdered liver is the pressor substance because he found, (a) administration of dried muscle to three rabbits to have no noteworthy effect and (b) cholesterol in oil fed to two rabbits (1 gram twice a week for nine and twelve weeks respectively) to cause an increase in blood pressure of about 50 per cent.

Using Van Eweyk and Schmidtmann's[6] method Schönheimer[4] also recently reported an increase in blood pressure of about 32 mm. mercury (from normal of 80 to 112) following administration of cholesterol in oil to several rabbits.

The method of Van Eweyk and Schmidtmann is, we believe, open to the following criticisms:

1. The rabbit must be fastened to a board. This entails a certain amount of struggling which may increase the blood pressure.

2. An incision through the skin alters the blood flow to the part which, in turn, influences the blood pressure as determined in that portion of the limb at that time.

3. It would be difficult, simultaneously, to record the manometer reading and observe the first appearance of blood in the incised area.

If by a more reliable method, free from the objections offered above, the findings of Fahr, Schmidtmann and the others could be confirmed, some additional light might be shed on the age-old controversy of the relationship between hypertension and the development of atherosclerosis.

In 1923, Anderson[7] described a simple apparatus for determining the systolic blood pressure in the central artery of the rabbit's ear. It occurred to us that by this means we could study the blood pressures of rabbits over long periods of time. Using Anderson's method, we have studied the systolic blood pressures of seven rabbits which were being fed lanolin for the production of

TABLE I

RABBIT NO. SEX	AGE AT DEATH (DAYS)	PREVIOUS OPERATIVE PROCEDURES	PERIOD LANOLIN FEEDINGS (DAYS)	NO. OF READINGS	SYSTOLIC BLOOD PRESSURE			DEGREE ATHEROSCLEROSIS	REMARKS
					HIGHEST	LOWEST	AVERAGE		
386 F	828*	Rt. & Lt. Suprarenalectomy	110	6	100	80	87	Slight	Readings 3 mo. after operations
408 M	664	Thyroidectomy Rt. & Lt. Suprarenalectomy	268	5	95	81	86	Marked	Readings before and 50 days after operations
416 F	573	Splenectomy	173	5	105	85	92	Marked	Readings 6 mo. after operations
417 F	603	Rt. & Lt. Suprarenalectomy	272	5	105	80	91	Marked	Readings before and 50 days after operations
423 M	482	Thyroidectomy	183	2	85	80	83	Moderate	Readings 3 mo. after operations
451 F	591	Splenectomy Rt. & Lt. Suprarenalectomy	100	5	100	80	87	Slight	Readings 4 mo. after operations
454 M	533	Splenectomy Rt. & Lt. Suprarenalectomy	106	6	100	88	90	Slight	Readings 4 mo. after operations
377 F	850*	Rt. & Lt. Suprarenalectomy	100	3	97	87	92	None	Readings 6 mo. after operations
490 M	512	Rt. & Lt. Suprarenalectomy	100	3	105	90	96	None	Readings 6 mo. after operations
441 F	631	Thyroidectomy Rt. & Lt. Suprarenalectomy	50	5	100	87	90	None	Readings 3 mo. after operations
463 M	419	Rt. & Lt. Suprarenalectomy	60	5	105	82	92	None	Readings 3 mo. after operations
411 F	603	Rt. & Lt. Suprarenalectomy	10	4	102	82	92	None	Readings before operations
414 F	647	Rt. & Lt. Suprarenalectomy	10	3	95	80	88	None	Readings before operations
374 M	629	Rt. & Lt. Suprarenalectomy	45	6	98	65	77	None	Readings 3 mo. after operations
497 M		Rt. & Lt. Suprarenalectomy	None	4	105	95	98	None	Readings before operations
432 F		None	None	3	98	87	93	None	
499 M	145	None	None	3	85	75	80	None	
501 M	535	Snip Thyroid removed	None	4	91	85	88	None	

*Approximate.

atherosclerosis, and of a number of control rabbits kept under similar hygienic and dietary conditions. Among these controls were rabbits which were subjected to the same operative procedures as the above seven, and some which were fed lanolin for varying periods of time from 10 to 100 days, but which at autopsy showed no atherosclerosis.

The blood pressure readings were made at irregular intervals during the course of the experiments. In order to avoid personal bias, the readings were made and recorded before the number of the rabbit was identified. The results of these blood pressure readings are summarized in Table I.

<center>DISCUSSION</center>

The average systolic blood pressure in the central artery of the rabbit's ear, according to Anderson,[7] varies between 75 and 90 mm. mercury. Our findings in rabbits, which we used as controls, agree with those of this investigator.

In each of the seven rabbits with atherosclerosis the average of all the readings fell within normal limits. The average blood pressure of these rabbits was 88, while in the 11 control rabbits it was 89.

It is true that the animals with marked atherosclerosis occasionally gave slightly higher readings than the controls. We cannot, at present ascribe any importance to this, although the possibility must be pointed out that experiments carried out over longer periods of time than ours, might show more definite rises. The point we wish to emphasize is, that although there was no significant hypertension at the end of the experimental periods, the vascular lesions at autopsy were in each case marked. The blood cholesterol was elevated in each of our animals fed with lanolin, increasing above normal to as high as 400 per cent.

We have, therefore, been unable to confirm Fahr, Van Leersum, Schmidtmann and Schönheimer, that feeding rabbits with cholesterol-containing substances, over long periods of time, causes elevations in blood pressure. The method used for the determination of the blood pressure is of course of the greatest importance. All three of the methods used by these investigators are open to serious criticism as has been pointed out above. For example, in the most direct of these methods, that of Van Leersum, alterations in the compressibility of the exposed carotid artery would be in favor of a higher blood pressure. By the method of Anderson, which we used, no surgical procedure is necessary, nor is it required to subject any part of the rabbit to unphysiologic conditions to obtain the readings.

The conception of atherosclerosis supported by Aschoff[8] and his pupils, especially Ranke,[9] is based on the older views of Virchow. This hypothesis assumes that certain mechanical influences and the processes of "wear and tear" play essential rôles. The primary anatomic change in the vessel wall, according to the Aschoff school, is a loosening of the intercellular cement substance of the intima and a deposition of fat and cholesterol esters within it. As the process continues, the deposition eventually involves all the layers of the intima, both cellular and interstitial elements.

The prevailing view, therefore, is that atherosclerosis is dependent upon certain mechanical factors, among which is increased vascular tension, which bring about loosening of the intimal cement substances and thus allows the deposition of fats and lipoids. Our observations on the blood pressure during the development of atherosclerosis in rabbits suggest that increase in blood pressure is not an essential factor in the deposition of lipoids in the intima.

CONCLUSIONS

1. The systolic blood pressure in the central artery of the rabbit's ear averages between 75 and 90 mm. mercury as reported by Anderson.

2. During the developmental stage of experimental lanolin atherosclerosis in rabbits, there is no significant hypertension.

3. It is believed that hypertension is not essential for the development of this type of atherosclerosis in rabbits.

We wish to thank Dr. David Marine for his helpful criticism in this work.

REFERENCES

[1]Fahr, Th.: Beiträge zur experimentellen Atherosklerose unter besonderer Berücksichtigung der Frage nach dem Zusammenhang zwischen Nebenniererenveränderungen und Atherosklerose, Verhandl. d. deutsch. path. Gesellsch., 1912, xv, 234.

[2]Van Leersum, E. C.: Alimentäre Blutdruckerhöhung, Ztschr. f. exper. Path. u. Therap., ii, 1912, p. 408.

[3]Schmidtmann, M.: Experimentelle Studien zur Pathogenese der Arteriosklerose, Virchow's Arch. f. path. Anat., 1922, ccxxxvii, 1.

[4]Schönheimer, R.: Über die experimentelle Cholesterinkrankheit der Kaninchen, Virchow's Arch. f. path. Anat., 1924, ccxlix, 1.

[5]Van Leersum, E. C.: Eine Methode zur Erleichterung der Blutdruckmessung bei Tieren, Pflüger's Arch. f. d. ges. Physiol., 1911, cxlii, 377.

[6]Van Eweyk, C., and Schmidtmann, M.: Zur Methodik der Blutdruckmessungen beim Kaninchen, Virchow's Arch. f. path. Anat., 1922, ccxxxvi, 420.

[7]Anderson, H. C.: Demonstration of Instrument for Taking Repeated Blood Pressures in Rabbits, Proc. Soc. Exper. Biol. and Med., 1923, xx, 295.

[8]Aschoff, L.: Virchow's Lehre von den Degenerationen (passiven Vorgängen) und ihre Weiterentwicklung, Virchow's Arch. f. path. Anat., 1921, ccxxxv, 152.

[9]Ranke, Otto: Über die Änderung des elastischen Wederstandes der Aortenintima und ihre Folgen für die Entstehung der Atheromatose, Beitr. z. path. Anat. u. z. allg. Path., 1923, lxxi, 78.

FURTHER EXPERIENCES WITH THE VERNES TEST FOR THE DIAGNOSIS OF SYPHILIS*

By Leon H. Cornwall, M.D., New York

FOUR years ago, in a brief report, the work of Arthur Vernes which culminated in his new seroreaction for syphilis was reviewed by Cornwall and Aronson,[1] and in a subsequent communication[2] the results of 850 examinations of blood and spinal fluid from a series of controlled clinical cases were reported.

This test is based upon the observation that syphilitic serums when added in certain proportions to a colloidal solution produce flocculation, whereas normal serums do not. In 1917, Vernes published his observations concerning this phenomenon.[3] Since that time several methods for the diagnosis of syphilis based upon flocculation or precipitation have been reported, notably that of Sachs-Georgi in Germany (1918)[4] and Kahn in this country (1922).[5]

An alcoholic extract of horse heart is the colloid that is employed. In Vernes' first method, the flocculation was read indirectly by means of a hemolytic indicator consisting of sheep cells and pork serum. The details of this technic may be found in my publication of 1922.[2] Since that time the technic of the test has been modified twice, with a gain in simplicity each time.

The second method dispensed with the indirect method of attaining the result. Inactivated serum was added directly to the colloidal suspension (alcoholic extract of horse heart) in the proportion of two parts of serum to one part of the extract diluted 1 to 6.5. After incubation at 20° C. for four hours the turbidity of this mixture was determined with the photometer of Vernes and Bricq. A control tube containing the same amount and dilution of serum but without any colloidal suspension was treated in the same manner. The end-result was represented by the difference between these two readings. (Turbidity of serum + colloid − turbidity of serum only = result.)

The photometer of Vernes and Bricq was equipped with an arbitrary numerical scale. By making a large number of quantitative estimations of the weight of the flocculi in suspension, weight-volume equivalents were determined for the numbers of the photometric scale. By means of a key furnished with each instrument, therefore, the numerical readings could be translated into terms of milligrams of precipitate per cubic centimeter.

This second method was designated by Vernes as the "Mesure Ponderale de la Floceulation."[6] It was soon superceded, however, by a third method as a result of an improved photometer devised by Vernes, Bricq and Yvon in 1922.[7]

This new instrument made possible a rapid and precise measurement of

From these findings, it was concluded that if the antigens were loosely bound to the cells or were a component of the ectoplasm, they would be more apt to be found in greater concentration in an aqueous solution obtained by merely washing the organisms grown on solid media than if an attempt were made to extract them by chemical or physical means.

In an attempt to prove this point washings were prepared as follows: The organisms were grown on solid media for twenty-four hours, taken off in salt solution, shaken in mechanical shaker for a few minutes and then immediately

TABLE I

TEST OF THE ANTIGENIC VALUE OF BROTH CULTURES OF VARIOUS ORGANISMS CONTAINING
1000 MILLION ORGANISMS PER C.C.

ANTIGENS	TITER OF IMMUNE SERA		
METHOD OR PREPARATION	AGGLUTIN-ATION	COMPL.-FIX.	BACTERIO-TROPIN
Broth culture of B. typhosus (1000 million organisms per c.c.)	1:18000	1:10000	1:2800
Centrifugate from broth culture	1:16000	1:10000	1:6000
Sediment from broth culture diluted to original volume	1:4000.	1:700	1:450
Broth culture of B. coli	1:18000	1:150	
Centrifugate from broth culture	1:12000	1:600	
Sediment from broth culture diluted to original volume	1:900	1:200	
Broth culture of pneumococcus Type I	1:80	0	
Centrifugate from broth culture	1:70	0	
Sediment from broth culture diluted to original volume	0	0	
Broth culture of streptococcus	1:500	1:10	
Centrifugate from broth culture	1:600	1:10	
Agar suspension (1000 million organisms per c.c.)	1:800	1:60	
Agar washings	1:800	1:50	
Broth culture of gonococcus	1:400	1:10	
Centrifugate from broth culture	1:400	1:10	
Agar suspension of equal number of organisms	1:500	1:100	
Agar washings	1:600	1:100	

TABLE II

TEST OF THE INFLUENCE OF THE INCUBATION TIME, PEPTONE AND SALT UPON BROTH
CULTURES OF B. COLI

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Plain Broth		
Incubated 5 hours (centrifugate)	1:150	1:10
" 24 hours "	1:8000	1:600
" 5 days "	1:300	1:50
" 10 days "	1:300	1:50
Plain broth without peptone		
Incubated 5 hours (centrifugate)	1:60	1:40
" 24 hours "	1:150	1:40
" 5 days "	1:60	1:10
" 10 days "	1:250	1:10
Plain broth without peptone and salt		
Incubated 5 hours (centrifugate)	1:40	1:15
" 24 hours "	1:40	1:10
" 5 days "	1:200	1:10
" 10 days "	1:80	1:15

passed through a Sharples centrifuge. The entire operation did not consume over half an hour from the time the growth was taken from the incubator to the final stage in the process.

These washings have been tested together with antigens prepared by other well-known methods and have been found to be extremely high in antigenic value. When injected into rabbits, following the method described above for testing broth cultures, the washings were found capable of producing antisera of very high titer. (See Tables III and IV.)

The washings have also been used with success in the rôle of antigens in the complement-fixation test. (See Table V.)

Recently[2] with these same washings prepared from the Streptococcus hemolyticus scarlatinae we have been able in collaboration with Dr. Pryer to produce a positive skin reaction in individuals susceptible to scarlet fever (Dick reaction), which reaction would become negative if the washings were

TABLE III

TEST OF THE ANTIGENIC VALUE OF VARIOUS ANTIGENS PREPARED FROM THE TYPHOID, PARA. A AND PARA. B, BACILLI DILUTED SO THAT ALL CONTAINED 1000 MILLION ORGANISMS PER C.C.

ANTIGENS		TITER OF IMMUNE SERA	
METHOD OF PREPARATION		AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Typhoid vaccine		1:9000	1:1000
Typhoid-paratyphoid vaccine comb.	B. typhosus	1:6000	1:100
	B. para. A.	1:5000	1:50
	B. para. B.	1:8000	1:250
Typhoid-paratyphoid washings	B. typhosus	1:16000	1:750
	B. para. A.	1:2000	1:750
	B. para. B.	1:20000	1:750
Typhoid-paratyphoid broth centrifugate	B. typhosus	1:10000	1:175
	B. para. A.	1:3000	1:60
	B. para. B.	1:12000	1:750
Typhoid glycerol vaccine	B. typhosus	1:6000	1:90
Typhoid serobacterin	B. typhosus	1:5000	1:75
Detoxicated T.A.B. vaccine	B. typhosus	1:2000	1:25
	B. para. A.	1:20	1:5
	B. para. B.	1:200	1:35
Residual T.A.B. vaccine	B. typhosus	1:2000	1:25
	B. para. A.	1:20	1:5
	B. para. B.	1:200	1:35

TABLE IV

TEST OF THE ANTIGENIC VALUE OF VARIOUS GONOCOCCUS ANTIGENS DILUTED SO THAT ALL CONTAINED THE SAME NUMBER OF ORGANISMS

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Agar washings of gonococcus	1:2000	1:100
Broth centrifugate of gonococcus	1:400	1:50
Gonorrhea (comb.) vaccine	1:900	1:90
Residual gonococcus vaccine	1:80	1:10
Gonococcus glycerol vaccine	1:100	1:70
Gonococcus serobacterin	1:30	1:50

TABLE V

TEST OF VARIOUS TYPHOID ANTIGENS WHEN USED AS SUCH IN THE COMPLEMENT-FIXATION TEST AGAINST A KNOWN ANTITYPHOID SERUM

ANTIGENS	IMMUNE SERA
METHOD OF PREPARATION	HIGHEST DILUTION FIXING COMPLEMENT
Mixture of broth centrifugate and agar washings of B. typhosus	1:90
Agar washings of B. typhosus	1:80
Agar washings of typhoid-paratyphoid mixture	1:60
Broth centrifugate of typhoid-paratyphoid mixture	1:90
Broth centrifugate of B. typhosus	1:70
Autolysates of B. typhosus	0

TABLE VI

TEST TO DETERMINE THE INFLUENCE OF REPEATED WASHING UPON THE ANTIGENIC VALUE OF SUSPENSIONS MADE FROM THE WASHED ORGANISMS. B. TYPHOSUS WAS USED AS THE TEST ORGANISM. 1000 MILLION ORGANISMS PER C.C.

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTINATION	COMPLEMENT FIXATION
Agar suspension	1:8000	1:325
Agar washing	1:11000	1:500
Washed organisms	1:4000	1:600
Broth growth	1:7000	1:300
Broth centrifugate	1:8000	1:250
Broth sediment	1:35000	1:600
Broth sediment washed	1:3000	1:500
Washings from antigen No. 63	1:1500	1:80

mixed with convalescent scarlet fever serum, similar to the results obtained by the Dicks of Chicago,[3] Zingher of New York,[4] Branch and Edwards,[5] Gatewood[6] and others with the Dick toxin.

Returning to the sedimented bacteria from which the washings were obtained, it was found, as with the sediment from the broth cultures in the first experiment, that the washed organisms were relatively low in antigenic properties. These sedimented bacteria washed the second time have yielded antigens even lower in antigenic properties than the sediment after the first washing and the second washings from this sediment have also suffered more or less in antigenic properties. (See Table VI.)

In carrying this work further, autolysates were prepared from the organisms, both before and after being washed, and it was found that they were even lower in antigenic value than the second washings from the unautolyzed sediment. This was especially marked of the autolysates from the washed organisms, showing that washings did not depend upon the autolyzation of the organisms for their antigenic properties. (See Table VII.)

Another experiment, which proves conclusively the antigenic value of these washings and centrifugates and would also tend to lend some weight to the suggestion that antigens are nonprotein in nature, was carried out by precipitating several different washings and broth centrifugates with various substances until a protein-free reaction was produced. These apparently protein-

TABLE VII

TEST TO DETERMINE THE INFLUENCE OF WASHING ORGANISMS, BOTH WITH AND WITHOUT LONG SHAKING, UPON THE ANTIGENIC VALUE OF THE AUTOLYSATES OF THE SEDIMENTED BACTERIA. SUSPENSION OF B. TYPHOSUS WAS USED, 1000 MILLION BACTERIA PER C.C.

ANTIGENS	TITER OF ANTISERA	
METHOD OF PREPARATION	AGGLUTIN-ATION	COMPLE-MENT FIX-ATION
Agar washings	1:12000	1:750
Broth centrifugate	1:12000	1:375
Autolysates from residue of agar washings	1:3000	1:100
Autolysates from residue of broth centrifugate	1:7000	1:500
Agar washings after shaking suspension 24 hr.	1:5000	1:500
Autolysates from residue of previous antigen	1:2000	1:100
Autolysates from agar growth not previously extracted by shaking	1:7000	1:150

free antigens were injected into rabbits, according to the usual method, and the serums tested for the presence of antibodies with positive results in several cases. (See Table VIII.)

From these experiments, it was suggested that the antigens of the organisms studied were more liable to be ectoplasmic than endoplasmic in origin, and for that reason this type of an antigen has been termed an "ectoantigen."

The more recent work which we wish to report at this time includes experiments carried on with washings or ectoantigens prepared from Type I pneumo-

TABLE VIII

TEST OF THE ANTIGENIC VALUE OF ANTIGENS APPARENTLY PROTEIN FREE

ANTIGENS			TITER OF IMMUNE SERA	
ORGANISMS	METHOD OF PREPARATION	METHOD OF PRECIPITA-TION	AGGLUTINA-TION	COMPLE-MENT FIX-ATION
B. typhosus	Washings	Phosphotungstic acid	1:200	1:25
B. typhosus	Broth centrifuge	Uranium acetate	1:80	1:25
B. coli	Washings	Acetone	1:100	1:5
B. coli	Broth centrifuge	Uranium acetate	1:200	1:50
B. coli	Broth centrifuge	Phosphotungstic acid	1:2000	1:75
Pneumococcus	Phenolized centrifugate	Acetone	1:40	1:10
Pneumococcus	Agar washings	Acetone	1:80	1:25

coccus. The organism used for all the experiments was a strain obtained from the Hygienic Laboratory, Washington, for the preparation of Type I antipneumococcic serum. It has repeatedly been tested, and according to all known serologic tests, has proved to be true to type with a minimum fatal dose to white mice of from one hundred millionth to one billionth cubic centimeter of a twenty-four hour broth culture.

In preparing the washings the seed cultures were grown on blood agar and transplanted to large flasks of ascites agar. These were incubated twenty-four hours and washed off with salt solution and divided into five lots.

TABLE IX

TEST TO DETERMINE THE VALUE OF TYPE I PNEUMOCOCCUS WASHINGS BY MEANS OF ACTIVE PROTECTION TESTS ON WHITE MICE

LOT	MOUSE	DILUTION OF CULTURE	RESULTS
I	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
II	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
III	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
IV	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	"
	5	0.001	Died
	6	0.01	"
	7	0.1	"
	8	1.	"
V	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	"
	5	0.001	"
	6	0.01	Died
	7	0.1	"
	8	1.	"

M. F. D. of culture—0.000000001 c.c.

Lot I. The suspension was subjected to a temperature of 60° C. for one hour, after which it was treated by the usual method for the preparation of washings.

Lot II. This was washed first and then the washings were subjected to a temperature of 50° C. for one hour.

Lot III. This was treated as Lot II but heated only to 40° C. for one hour.

Lot IV. This suspension was treated with 0.3 per cent cresols and allowed to stand twenty-four hours before treatment as a washing.

Lot V. This was treated at once without heat or cresols, according to the previously described method of preparing washings.

To all of the final products except Lot IV, 0.3 per cent cresols were added as a preservative. White mice were given two doses of 0.5 c.c. of these extracts

subcutaneously one week apart and one week after the last dose were injected intraperitoneally with varying doses of the live organisms. (See Table IX.)

Results.—The results showed that Lot I which had been heated to 60° C. for one hour before being washed protected against 10,000 M. F. D. and the same was true of Lots II and III which had been heated to 50° C. and 40° C., respectively. Lot IV which had been sterilized by the addition of 0.3 per cent cresols for twenty-four hours before washing, protected against 100,000 M. F. D. while Lot V extracted a few minutes with salt solution, protected against at least 1,000,000 M. F. D.

Discussion.—From these results it is evident that an aqueous extract of Type I pneumococcus may be obtained from the washings of the organisms, fairly stable at a temperature of 60° C. for one hour with antigenic properties extremely high, without resorting to chemical extraction; and suggests the possibility of the use of such an extract for prophylactic purposes against pneumonia in the human.

REFERENCES

[1]Ferry and Fisher: Brit. Jour. Exper. Path , 1924, v, 185 and 205.
[2]Ferry, Pryer and Fisher: To be published.
[3]Dick and Dick: Jour. Am. Med. Assn., 1924, lxxxii, 265.
[4]Zingher: Ibid., 1924, lxxxiii, 432.
[5]Branch and Edwards: Ibid., 1924, lxxxii, 1260.
[6]Gatewood: Ibid., 1924, lxxxiii, 494.

COMPLEMENT FIXATION IN TUBERCULOSIS WITH PARTICULAR REFERENCE TO A GROUP OF 102 POSITIVE REACTIONS[*]

By Lloyd R. Jones, U. S. Veterans' Hospital No. 98, Castle Point, New York

DURING the past few years it has been the purpose of this laboratory to study the complement-fixation reaction as applied to the blood sera of patients, in the diagnosis of tuberculous disease. It is recognized that the test has peculiar limitations and the purpose of this study has been to determine the specificity of a positive reaction. The problem has been attacked from the viewpoint of determining the value of the positive reaction as obtained from the blood serum.

With one particular lot of antigen, which after extensive trial, with many tuberculous and clinically nontuberculous sera, was found to possess desirable antigenic properties, the blood sera of 617 patients were subjected to the test. Of the number tested, 102 gave positive reactions of either "++" or "+++" degree (basis: four plus representing complete inhibition of hemolysis).

Of the more than 400 patients giving a negative reaction to the test, it was recognized that many of the number were tuberculous. However, the purpose

of the present discussion is merely to determine the incidence of tuberculous disease among those individuals giving a positive reaction.

The technic of the reaction as made in this particular study was as follows:

a. *Antigen.*—Necessarily an essential element in making a satisfactory test is a reliable antigen of established antigenic properties. Several strains of B. tuberculosis were grown in flasks of nutrient glycerin bouillon in large volume, then removed and washed with saline solution, centrifuged and later removed to a drying chamber. Masses of the partially dried bacilli were then transferred to a mortar and ground with a pestle for more than three hours, only slight amounts of distilled water being added. Smears made of the material and stained with fuchsin showed considerable disintegration of many bacillary bodies. After the grinding process, distilled water was added in amount sufficient to make the resulting mixture markedly opaque. One half of one per cent of phenol was added as a preservative and the mixture heated in a water-bath to 70° C. for twenty minutes. For use, one to ten dilutions were made in physiologic saline solution and standardization effected by titration with known positive and negative sera. The dose of antigen for use in the test was considered as an amount two times the least amount giving complete inhibition with a positive serum, in the event that twice that tentative amount did not inhibit hemolysis with a negative serum. Lots of antigen showing excessive anticomplementary or irregular antigenic properties were discarded.

b. *Patient's Serum.*—Clear blood serum was regularly inactivated at 56° C. for thirty minutes on the day following withdrawal.

c. *Complement.*—Pooled blood sera from two or more guinea pigs. The serum from each pig having been previously tested for fixability with this antigenic system. For use, one to ten dilutions were made in physiologic saline solution.

d. *Hemolytic Amboceptor.*—Blood serum of a rabbit highly immunized to sheep erythrocytes.

e. *Sheep Cells.*—Obtained from the sheep and used as a 5 per cent suspension of the washed cells.

The tests were performed in a manner closely paralleling the classical Wassermann test. Two units of complement, the dose of antigen and 0.2 c.c. of patient's serum were incubated for one hour in the water-bath at 37° C., whereupon two units of hemolytic amboceptor and 0.5 c.c. of the cell suspension were added to each tube and readings made after a thirty minute period of secondary incubation. Concerning the length of the primary incubation period, it was noted that a greater degree of specific fixation was obtained with an incubation period of one hour at 37° C. than was obtained at "ice box" temperature (6° C.) for four hours, a distinct variation from the experience frequently encountered in working with bacillary antigens.

An attempt was made to classify the various physical findings on the 102 patients giving a "+++" or "++++" reaction to the test. Competent examiners will frequently disagree on a diagnosis of clinical tuberculosis; hence, the clinical diagnoses as listed cannot be considered as an absolute index to the accuracy of the test. Data considered in basing a diagnosis of clinical tuberculosis were obtained from the history, temperature and weight charts, physical examinations, sputum examinations, Roentgen plates and interpreted in manner as is customary among most authorities on tuberculosis.

Pulmonary tuberculosis, active, with B. tuberculosis present in the sputum.	Cases	55
Pulmonary tuberculosis, active clinically, well defined cases but with negative sputum.	Cases	22
Pulmonary tuberculosis quiescent clinically, well defined evidence of former tuberculous process	Cases	21
Pulmonary tuberculosis negative clinically. Syphilitic. Wassermann positive.	Cases	1
Pulmonary tuberculosis negative clinically. Diagnosis: Laryngitis	Cases	1
Pulmonary tuberculosis negative clinically. Diagnosis: Asthma	Cases	1
Pulmonary tuberculosis negative. Otherwise negative clinically	Cases	1

The results here given, though encouraging, are not of sufficient scope to prognosticate the value of the reaction. In surveying these cases the purpose has been to determine, in so far as is clinically possible, the incidence of definite tuberculous disease among individuals giving a positive reaction to the test.

Concerning the so-called "cross-fixation" or nonspecific fixation with luetic sera, frequently reported, it was noted in this investigation that ten of the individuals gave a positive Wassermann reaction concomitantly; however, in each case diagnoses of dual disease: viz., syphilis and tuberculosis were made in all except one, who was considered as being clinically tuberculosis-free. Of the ten cases referred to, five were available for specific therapy and after intensive antiluetic treatment, with the disappearance of Wassermann reaction, it was noted in all cases that positive reactions with the complement-fixation test for tuberculosis persisted. So it is thought that with the use of a properly prepared antigen and properly controlled technic, nonspecific fixations with luetic sera need not be frequently encountered.

In making the test with the blood sera of patients who were in a far advanced stage of tuberculosis, it was noted that negative reactions were obtained in approximately 50 per cent of such cases. Presuming that we are dealing with a true antibody reaction, this anomaly is readily explained by the supposition that enormous amounts of tubercle antigen, are elaborated into the blood stream, incident to the extensive tuberculous process in a terminal case. By absorption, neutralization or other physiochemical processes the "reaction-bodies" necessary for the fixation of complement are rendered inert, hence, when such a condition is established, negative complement-fixation reactions will follow.

SUMMARY

1. An antigen preparation for the test should be accepted only after thorough preliminary trial demonstrating satisfactory antigenic qualities. The amount to be used in the test can be determined only after careful experimenta-

tion and must be such as permits a very fine quantitative adjustment between complement, antigen and patient's serum.

2. Several control sera should be regularly employed to detect evidence of false fixation, antigen deterioration, etc.

3. Limited observations, herein reported, would tend to promote the belief that positive reactions as obtained with the test when correctly performed, indicate with a high degree of accuracy the presence of tuberculosis "reaction-bodies" irrespective of whether the disease is then clinically active or quiescent.

THE INFLUENCE OF EXPERIMENTAL ATHEROSCLEROSIS UPON THE SYSTOLIC BLOOD PRESSURE IN RABBITS*

BY SHEPARD SHAPIRO, M.D., AND DAVID P. SEECOF, M.D., NEW YORK CITY, N. Y.

INCREASES in systolic blood pressure have been reported in rabbits following the prolonged feeding of cholesterol in oil, or of cholesterol containing substances administered for the purpose of producing atherosclerosis, by Fahr,[1] Van Leersum,[2] Schmidtmann[3] and Schönheimer.[4]

Three different methods for measuring the blood pressure were used by these authors. The method employed by Fahr[1] is as follows: A rubber cuff connected to a manometer is placed around the abdomen of the rabbit; air is forced into this cuff until the abdominal aorta is occluded, as indicated by the disappearance of the femoral pulse. The pressure in the cuff is then gradually released, and the instant the first femoral pulse returns the reading is taken and recorded.

In seven of the ten rabbits to which he had fed egg yolk and milk and which showed atherosclerosis at necropsy, Fahr observed increases in the blood pressure. In two of four rabbits which he had suspended, head down, daily, he observed similar elevations in blood pressure. The arteries in these latter four rabbits showed no atherosclerosis. Fahr observed cardiac hypertrophy in those rabbits in which he found hypertension following egg yolk feeding; and claims, further, that there was in these animals a parallelism between the degree of atherosclerosis, the cardiac hypertrophy, and the hypertension.

There is an obvious objection to Fahr's method. Constriction of the rabbit's abdomen is unphysiologic and *per se* gives rise to uncontrollable alterations in blood pressure.

Van Leersum[5] used a method which he published in 1911, and which briefly is as follows:

The carotid artery is isolated and a rubber tube passed around it. The wound is then permitted to heal with the rubber tube in position. At the time the reading is taken (from two to four weeks after the operation) a rubber cuff is joined to the tube and held in place by forceps. This cuff is connected to a

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rubber bulb and a manometer, and air is forced into the tube until the artery is occluded. The pressure is then gradually released and at the instant the first pulsation is felt by palpation of the vessel the reading is made. This is recorded as systolic pressure. By this means Van Leersum[2] observed an increase in systolic blood pressure of 30 per cent after feeding powdered liver to rabbits for long periods of time.

This method offers a very direct means for determining blood pressure and would be an excellent one, provided the artery could be thus isolated without altering the compressibility of its walls through inflammatory reactions. This objection seems to render the method both impractical and prohibitive.

Van Eweyk and Schmidtmann,[6] therefore, in 1922, published the following method:

The rabbit is fastened to a board. The circulation in one of the forelegs is occluded by means of a tourniquet. A rubber cuff, which is connected to a bulb and manometer, is then placed around the upper portion of the leg. Air is forced into the cuff until the manometer reads 160 mm. mercury. The tourniquet is then removed. A small incision through the skin is made below the point of occlusion and the pressure in the cuff is gradually reduced. When the blood appears in the small arteries of the exposed fascia, the manometer is read and this reading recorded. The incision is sewed up and iodine applied. Using this method, Schmidtmann[3] observed a rise in blood pressure following the feeding of powdered liver in six rabbits, confirming in this one respect Van Leersum. He reports, further, that the blood pressure fell after powdered liver was discontinued and rose again when feeding with liver was resumed.

At necropsy, Schmidtmann found intimal changes present in the aortas of these rabbits. It should be emphasized that Van Leersum, in the eight rabbits in which he observed elevations in blood pressure (as determined by his method) following feeding with powdered liver, failed in all cases to find atherosclerosis on postmortem examination. Schmidtmann also claims that there is a direct relationship between the severity of the vascular lesion and the height of the blood pressure; and concludes that the cholesterol contained in the powdered liver is the pressor substance because he found, (a) administration of dried muscle to three rabbits to have no noteworthy effect and (b) cholesterol in oil fed to two rabbits (1 gram twice a week for nine and twelve weeks respectively) to cause an increase in blood pressure of about 50 per cent.

Using Van Eweyk and Schmidtmann's[6] method Schönheimer[4] also recently reported an increase in blood pressure of about 32 mm. mercury (from normal of 80 to 112) following administration of cholesterol in oil to several rabbits.

The method of Van Eweyk and Schmidtmann is, we believe, open to the following criticisms:

1. The rabbit must be fastened to a board. This entails a certain amount of struggling which may increase the blood pressure.

2. An incision through the skin alters the blood flow to the part which, in turn, influences the blood pressure as determined in that portion of the limb at that time.

3. It would be difficult, simultaneously, to record the manometer reading and observe the first appearance of blood in the incised area.

If by a more reliable method, free from the objections offered above, the findings of Fahr, Schmidtmann and the others could be confirmed, some additional light might be shed on the age-old controversy of the relationship between hypertension and the development of atherosclerosis.

In 1923, Anderson[7] described a simple apparatus for determining the systolic blood pressure in the central artery of the rabbit's ear. It occurred to us that by this means we could study the blood pressures of rabbits over long periods of time. Using Anderson's method, we have studied the systolic blood pressures of seven rabbits which were being fed lanolin for the production of

TABLE I

RABBIT NO. SEX	AGE AT DEATH (DAYS)	PREVIOUS OPERATIVE PROCEDURES	PERIOD LANOLIN FEEDINGS (DAYS)	NO. OF READINGS	SYSTOLIC BLOOD PRESSURE			DEGREE ATHEROSCLEROSIS	REMARKS
					HIGHEST	LOWEST	AVERAGE		
386 F	828*	Rt. & Lt. Suprarenalectomy	110	6	100	80	87	Slight	Readings 3 mo. after operations
408 M	664	Thyroidectomy Rt. & Lt. Suprarenalectomy	268	5	95	81	86	Marked	Readings before and 50 days after operations
416 F	573	Splenectomy	173	5	105	85	92	Marked	Readings 6 mo. after operations
417 F	603	Rt. & Lt. Suprarenalectomy	272	5	105	80	91	Marked	Readings before and 50 days after operations
423 M	482	Thyroidectomy	183	2	85	80	83	Moderate	Readings 3 mo. after operations
451 F	591	Splenectomy Rt. & Lt. Suprarenalectomy	100	5	100	80	87	Slight	Readings 4 mo. after operations
454 M	533	Splenectomy Rt. & Lt. Suprarenalectomy	106	6	100	88	90	Slight	Readings 4 mo. after operations
377 F	850*	Rt. & Lt. Suprarenalectomy	100	3	97	87	92	None	Readings 6 mo. after operations
490 M	512	Rt. & Lt. Suprarenalectomy	100	3	105	90	96	None	Readings 6 mo. after operations
441 F	631	Thyroidectomy Rt. & Lt. Suprarenalectomy	50	5	100	87	90	None	Readings 3 mo. after operations
463 M	419	Rt. & Lt. Suprarenalectomy	60	5	105	82	92	None	Readings 3 mo. after operations
411 F	603	Rt. & Lt. Suprarenalectomy	10	4	102	82	92	None	Readings before operations
414 F	647	Rt. & Lt. Suprarenalectomy	10	3	95	80	88	None	Readings before operations
374 M	629	Rt. & Lt. Suprarenalectomy	45	6	98	65	77	None	Readings 3 mo. after operations
497 M		Rt. & Lt. Suprarenalectomy	None	4	105	95	98	None	Readings before operations
432 F		None	None	3	98	87	93	None	
499 M	145	None	None	3	85	75	80	None	
501 M	535	Snip Thyroid removed	None	4	91	85	88	None	

*Approximate.

atherosclerosis, and of a number of control rabbits kept under similar hygienic and dietary conditions. Among these controls were rabbits which were subjected to the same operative procedures as the above seven, and some which were fed lanolin for varying periods of time from 10 to 100 days, but which at autopsy showed no atherosclerosis.

The blood pressure readings were made at irregular intervals during the course of the experiments. In order to avoid personal bias, the readings were made and recorded before the number of the rabbit was identified. The results of these blood pressure readings are summarized in Table I.

<div align="center">DISCUSSION</div>

The average systolic blood pressure in the central artery of the rabbit's ear, according to Anderson,[7] varies between 75 and 90 mm. mercury. Our findings in rabbits, which we used as controls, agree with those of this investigator.

In each of the seven rabbits with atherosclerosis the average of all the readings fell within normal limits. The average blood pressure of these rabbits was 88, while in the 11 control rabbits it was 89.

It is true that the animals with marked atherosclerosis occasionally gave slightly higher readings than the controls. We cannot, at present ascribe any importance to this, although the possibility must be pointed out that experiments carried out over longer periods of time than ours, might show more definite rises. The point we wish to emphasize is, that although there was no significant hypertension at the end of the experimental periods, the vascular lesions at autopsy were in each case marked. The blood cholesterol was elevated in each of our animals fed with lanolin, increasing above normal to as high as 400 per cent.

We have, therefore, been unable to confirm Fahr, Van Leersum, Schmidtmann and Schönheimer, that feeding rabbits with cholesterol-containing substances, over long periods of time, causes elevations in blood pressure. The method used for the determination of the blood pressure is of course of the greatest importance. All three of the methods used by these investigators are open to serious criticism as has been pointed out above. For example, in the most direct of these methods, that of Van Leersum, alterations in the compressibility of the exposed carotid artery would be in favor of a higher blood pressure. By the method of Anderson, which we used, no surgical procedure is necessary, nor is it required to subject any part of the rabbit to unphysiologic conditions to obtain the readings.

The conception of atherosclerosis supported by Aschoff[8] and his pupils, especially Ranke,[9] is based on the older views of Virchow. This hypothesis assumes that certain mechanical influences and the processes of "wear and tear" play essential rôles. The primary anatomic change in the vessel wall, according to the Aschoff school, is a loosening of the intercellular cement substance of the intima and a deposition of fat and cholesterol esters within it. As the process continues, the deposition eventually involves all the layers of the intima, both cellular and interstitial elements.

light absorption (turbidity) and its direct expression in terms of optic density. The numbers of the scale correspond to degrees of optic density.

The results reported herein were obtained with the last method. The work has been done in the Neurological Department of the College of Physicians and Surgeons, Columbia University and was made possible by a grant from the Commonwealth Fund.

TECHNIC

1. EXTRACT OF HORSE HEART (PERETHYNOL).—The muscular part of a fresh horse heart is finely chopped or ground and the pulp obtained is dehydrated by maceration in 95 per cent alcohol. It is allowed to stand for about one hour, being occasionally stirred. At the end of that time the alcohol is partially expressed and the dehydration is terminated by thoroughly mixing the pulp and alcohol in a mortar. The alcohol is then removed by expression and the pieces of tissue are spread out in a thin layer on glass plates and dried
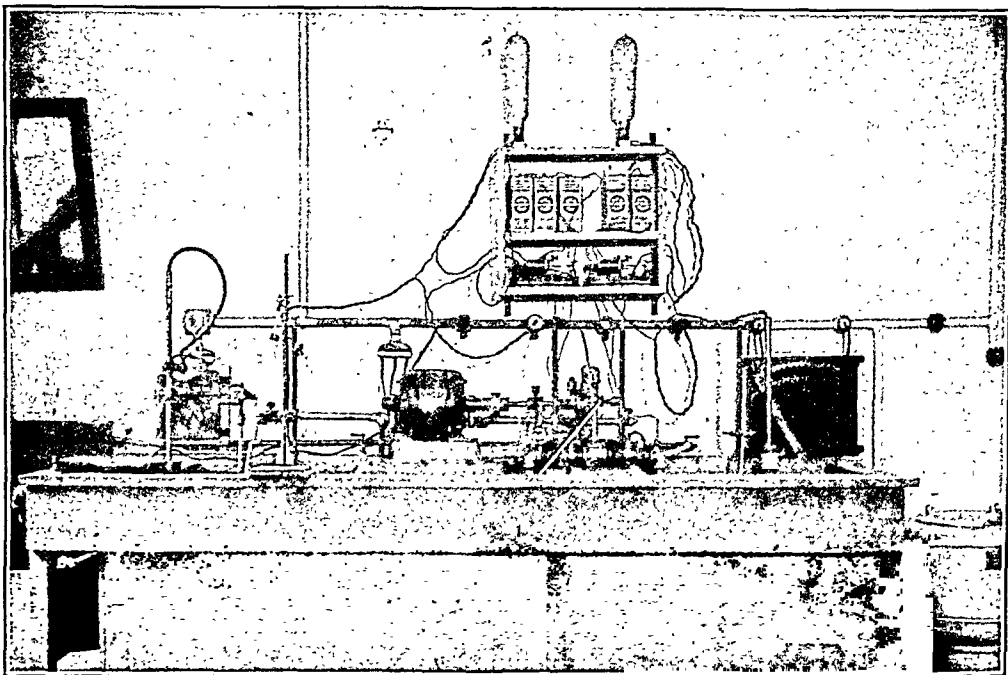
Fig. 1.—Water-baths built in a table. The stirring apparatus and delivery pipette are at the left. The centrifugal pump is in the middle background.

in the thermostat at 37° C. for twenty-four hours. The dried pulp is then finely pulverized in a mortar.

To 30 gm. of the powdered heart are added 60 gm. of sand, previously washed in alcohol and dried, and 250 c.c. of ethylene perchloride, the boiling point of which is from 115° to 121° C. This is placed in a 500 c.c. distilling flask which is connected with a Sohxlet extracting apparatus and condenser. The extraction apparatus is arranged so that the distillation may be conducted under partial vacuum by means of an air or a water pump. The distilling flask is heated in a water-bath, the temperature of which should be maintained at from 60° to 65° C. The operation is conducted at a pressure of 4 cm. of mercury so that the temperature in the distilling flask does not exceed 35° C. This requires from six to seven hours (forty siphonnements).

The powdered residue is again dried at 37° C. and to it are added 200 c.c. of absolute alcohol. Distillation is again accomplished by means of the same apparatus at a pressure

of from 5 to 6 cm. of mercury. The temperature of the water-bath should be from 60° to 65° C. and the temperature of the contents of the flask 30° C. The operation requires five hours (thirty siphonnements).

The residue is discarded and the final distillate is allowed to stand for twenty-four hours and then filtered. A small portion of this filtrate is dried at 60° C. for from eight to nine hours, and weighed. On the basis of this calculation, the alcoholic solution is adjusted so that it contains 15 gm. of the dried extract per liter. This is accomplished by the addition of more alcohol or by further evaporation in vacuum at 30° C. according to the indication.

2. COLLOIDAL SUSPENSION.—The extract of horse heart is diluted with doubly distilled water. If the extract is added, drop by drop, to the water, the latter being rapidly agitated during the process, the resulting suspension is pale and limpid because of the fine division of the molecules. If on the other hand distilled water is added, drop by drop to the extract, a milky suspension is obtained due to the large size of the molecules.

Because of the difference in the flocculent properties of blood serum and spinal fluid, colloidal suspensions of very different physical states are required. In each case the mix-
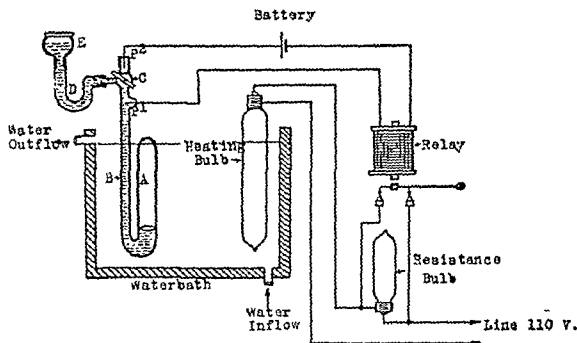
Fig. 2.—Diagram of water-bath with the electrical connections for heating and thermo regulation.

tures are made in a water-bath at the temperature of 25° C., all ingredients and glassware having been brought to this temperature before beginning the preparation of the suspension. During preparation, the ingredients are stirred by means of a mechanical apparatus having a glass propeller operated by an electric motor. The propeller is rotated at the rate of 200 revolutions per minute.

Preparation of Suspension, etc.

Vernes emphasized the necessity for uniformity of temperature during all stages of the procedure and his laboratory is kept at 26° C. It was impossible for us to accomplish this without constructing a thermostatic room which we were precluded from doing because of the expense.

It occurred to us that a water-bath of sufficient size for us to work in would meet the requirements, provided all tubes were immediately corked with rubber stoppers. In conferring with H. B. Williams of the Department of Physiology, we found that such a bath could be improvised without much difficulty or expense by adapting certain principles previously utilized by him in constructing a constant-temperature, water-cooled calorimeter. This improvised bath with the required attachments is illustrated in Figs. 1 and 2. The total cost did not exceed one hundred dollars.

. . . It consists of a metal water tank jacketed with wood. There is an air chamber of approximately two inches between the metal tank and wooden jacket on the sides and bottom. This air chamber is filled with lagging for the purpose of insulation. Ground cork or felt in suitable material for lagging. A perforated metal shelf was inserted 1½ inches below the level of the water. The ingredients and test tube racks are kept on this shelf immersed in water of the proper temperature from the beginning to the end of the test. Constant circulation of water from the city supply is maintained in this tank by means of a Viking centrifugal pump. During the summer season, if the temperature of the tap water exceeds 20° C., it may be cooled by allowing it to pass through coils immersed in a freezing mixture of ice and salt. To obviate rapid changes in the temperature of the bath the tank should have a depth of 14 to 16 inches. The water is heated to the desired temperature by means of an electric heating lamp of 250 watts. The temperature is controlled by means of a chloroform mercury thermostat previously described by Williams[8]. This is illustrated in Fig. 2. It consists of a U-shaped glass tube with one closed bulbous
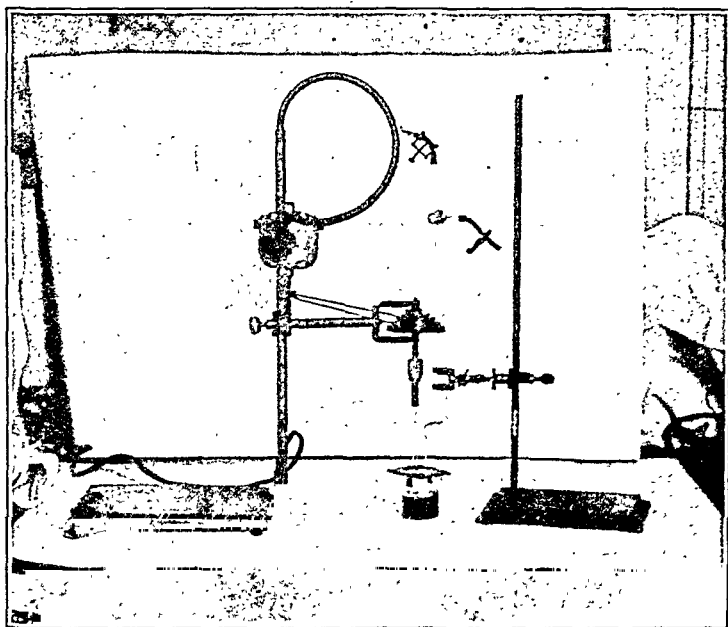
Fig. 3.—Stirring apparatus and delivery pipette employed for the preparation of the suspension.

arm, A. The other arm, B, is open and has a calibre of about 2 mm. Near the open end of B there is a three-way stopcock, C, and a lateral arm, D, communicating with the mercury reservoir, E. A is filled with chloroform and B with mercury from the reservoir. When the desired amount of mercury has been determined, communication between the reservoir, E, and B is closed by means of the three-way cock, C. A platinum wire, P^1, is fused into the side of the tube, B, and is always is contact with the mercury. Another platinum wire, P^2, projects into the upper open end of B through a small cork. The amount of mercury should be regulated so that, at the desired temperature, there is contact between it and the platinum tip, P^2, thus closing the relay circuit. When the relay is closed the heating and the resistance bulbs are in series, thus reducing the luminosity of the heating bulb. As soon as the mercury falls sufficiently to break its contact with P^2, the relay circuit is opened and the heating and resistance bulbs are parallel, causing the heating bulb to again become fully lighted. The low specific heat and high coefficient of cubical expansion of chloroform result in rapid changes in its volume with slight changes in the temperature of the water in the bath. This apparatus can be adjusted to maintain a constant temperature within a small fraction of a degree. We have found it extremely satisfactory.

Preparation of Suspension for Blood
Dilution 1/6.5

Place in a beaker of 45 mm. diam. doubly distilled water.	12.0 c.c.
Add extract drop by drop at the rate of 1 c.c. per minute.	6.0 c.c.
Add doubly distilled water	21.0 c.c.

Diameter of propeller 35 mm. The tip of delivery pipette should be about 3 cm. from surface of liquid.

Preparation of Suspension for Spinal Fluid
Dilution 1/4

Place in a beaker of 28-30 mm. diam. extract.	3.0 c.c.
Add doubly distilled water, drop by drop at the rate of 3 c.c. per minute.	9.0 c.c.

After 1/3 of the water has been added the remainder may be added *more rapidly.* Diameter of propeller 15 mm. The tip of delivery pipette should be in contact with surface of liquid.
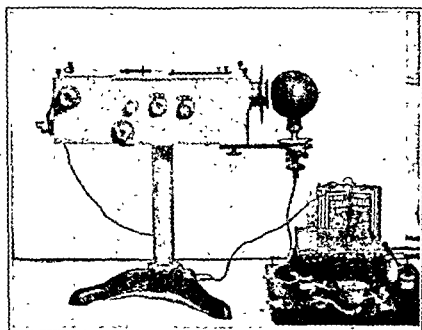
Fig. 4.—Photometer of Vernes, Bricq and Yvon

3. TEST.—Tubes of 60 by 13 mm. dimensions are used.

Blood

	(Test) Tube I	(Control) Tube II
	c.c.	c.c.
Serum-inactivated 20 minutes at 55° C.	0.8	0.8
Extract (Perethynol) 1/6.5	0.4	0.0
Absolute alcohol 1/6.5	0.0	0.4

Cork with rubber stoppers, shake, and place in water-bath for four hours at 25° C. Result: Optic density of Tube I minus Tube II.

Spinal Fluid

	Tube I (Test)	Tube II (Control)
	c.c.	c.c.
Spinal fluid	1.6	0.0
Extract (Perethynol) 1/4	0.4	0.4
Doubly distilled water	0.0	1.6

Five control tubes are prepared for each series of spinal fluid tests. The average optic density of these five tubes is taken as a control for the whole series. The tubes are corked with rubber stoppers, shaken and placed in the water-bath at 25° C. for four hours.

The optic density of turbid spinal fluid is read immediately and this result is subtracted from the final reading.

4. FINAL RESULT.—After incubation for four hours the tubes are inverted several times and the optic density of Tubes I and II determined. The optic density of Tube I minus the optic density of Tube II gives the result.

Before presenting the results of this reaction, it is necessary to explain the method that must be applied for its interpretation.

First, all blood serums and spinal fluids produce flocculation or precipitation under certain conditions.

Second, the precipitation caused by normal serum varies in different individuals just as the normal temperature or pulse rate varies in individual cases.

Third, the average mean flocculence of normal blood serum and spinal fluid has been established and the upper limit of flocculence in normals has been determined.

When successive examinations of normals are made and the results are charted graphically the curve of these results is approximately a horizontal line with only slight undulations. In this respect we may compare the graph to a temperature record of a normal individual. This is known as normal flocculence.

Syphilitic infection increases the flocculation. Syphilitic serum is spoken of, therefore, as hyperflocculent or surflocculent and the result of the reaction obtained therewith is spoken of as hyperflocculence or surflocculence.

Normal blood serums and spinal fluids show oscillations that are usually within narrow limits (blood serum 0 to 6, spinal fluid 0 to 30). In rare cases, the flocculence of a normal serum may reach 27 and a normal spinal fluid may reach 50.

The sensitiveness of the reaction has been adjusted so that normal flocculence is confined to the lower degrees of the scale.

A proper conception of this reaction requires a modification of the terminology that we have applied to serologic reactions.

Antigen is a biologic term signifying a substance that stimulates the formation of antibodies in the host. The extract used for this test is not an antigen. We may call it reagin if we choose. In reality it is a colloidal suspension possessing certain definite physicochemic properties. The reaction produced by the mixture of this suspension with blood serum or spinal fluid is a chemical or physicochemic one viz., flocculation or precipitation. This flocculation or precipitation is accompanied by a modification of the turbidity of the mixture. This modification of turbidity is expressed in terms of optic density, a factor that has a definite and well understood physical significance.

Optic density is a measure of light absorption or diffusion (logarithm of the incident minus the transmitted light). It is determined by means of the photometer previously alluded to.

In expressing the results of this reaction we must discard the terms "negative," "doubtful," "weakly positive," "positive," "strongly positive," etc., and substitute numerical terms of optic density. Failure to do this will retard the understanding of the reaction and defeat the object of its use. Except in rare instances it cannot furnish a diagnosis when the result is divorced from the clinical aspects of the case to which it is applied.

The reaction is designed to be correlated with clinical findings. The interpretation should be made by one who is in touch with all of the essential clinical facts.

In truth, no laboratory procedure is designed to diagnose, in disregard of or in ignorance of, clinical facts although unfortunately the laboratory is too frequently called upon to serve this function.

It should be emphasized that the interpretation of this procedure depends upon the curve obtained by successive examinations of the blood serum or spinal fluid. Its diagnostic significance depends upon the correlation of the results with clinical observations.

For example, a group of clinical facts present themselves in a given case that suggest the possibility of syphilitic etiology although no history of syphilitic infection is given nor are there any physical signs that give reasonable assurance of this. The Vernes result shows an optic density of zero. Can one postulate the absence of syphilis? No. No more surely than one can postulate the absence of hyperthermia with one record of a temperature of 98.6° F. One can only say that the flocculation in one case and the temperature in the other were within the range of normal at that particular time.

Most nonsyphilitic serums give a photometric reading of 0 to 6. An elevation to 27 in a presumably nonsyphilitic serum has been observed only once by Vernes in over 650,000 examinations. The reaction has been so regulated that the vast majority of nonsyphilitic serums give readings from 0 to 6 or 8 and the oscillation noted on successive examinations is usually confined within these limits. This characteristic is distinctive for nonsyphilitic serums. One can speak of complete absence of flocculation only when the photometric result is 0.

Results from 0 to 8 indicate the absence of syphilis in a vast majority of cases.

The original technic for spinal fluid was so regulated that nonsyphilitic fluids gave photometric readings as high as 30 and at least 50 was necessary to justify a diagnosis of meningeal syphilis on a single examination. Subsequent modification of the technic has provided the same limits for spinal fluids as for blood. *Our results were obtained with the earlier technic.*

As a therapeutic index, this test offers features of especial attraction. The graph of successive results displays, in an objective manner not possessed by other serologic reactions, the efficacy of the therapy upon the surflocculence. The photometric readings may descend to the nonsyphilitic zone but if the treatment has been insufficient, the curve will rise again.

The rise or fall or stability of the photometric readings indicates the progression, regression, or stationary character of the infection.

One more important point must be mentioned. This concerns the significance of a low photometric reading in a case of treated syphilis. Does this mean that the infection has been extinguished? In such a case Vernes recommends the administration of one or two doses of an arsenical. Following this, he advises monthly examinations of the blood for eight months and an examination of the spinal fluid at the eighth month. If the curve does not oscillate beyond the limits prescribed for nonsyphilitic serums during that period and the spinal fluid is negative at the eighth month, he states that he has never seen

a recurrence of the surflocculence in his experience which includes over 650,000 results. In case the infection has not been extinguished, the administration of arsenic will have a provocative action on the flocculation. Low flocculence or the absence of flocculence for eight months is not alone sufficient. This period must follow arsenical administration. This is spoken of as the "jalon arsenical," or postarsenical interval.

We have collected our records of the application of the Vernes and Wassermann tests on 1466 blood serums and spinal fluids from cases in the Neurological Department of the Vanderbilt Clinic. Our purpose was to make a comparative study of the relative interpretative value of the two tests rather than merely a numerical comparison of the results of the two tests.

Histories and clinical data have been carefully investigated in arranging the clinical groups. It would not have been difficult to multiply the number of results herein contained but mere numbers would fail to give an idea of the relative value of these tests for clinical application.

<center>BLOODS</center>

Twelve hundred and ninety-seven serums were examined.

The cases were divided into three clinical groups: nonsyphilitic, syphilitic, and miscellaneous.

<center>*I. Nonsyphilitic Group*</center>

The serums numbered 758.

The Wassermann reactions were negative in 735 and indeterminate in 23.

In the twenty-three serums that gave indeterminate Wassermann reactions the photometric readings were 0 to 7. Careful investigation of these cases failed to disclose anything suggestive of syphilis.

The Vernes photometric results were as follows:

<center>

0 to 4 in	634 serums
5 to 9 in	90 serums
10 to 20 in	31 serums
21 to 24 in	3 serums

758 serums

</center>

Of the three cases giving photometric readings above 20, two were psychoneurotics and one a mental defective. The thirty-one serums that gave photometric readings from 10 to 20 came from cases diagnosed as follows (page 839):

Only a very few serums in this group were examined more than once. In these, the photometric variation was from 0 to 3 points.

In this group of 758 serums from clinically nonsyphilitic cases, most of which were examined once only, the Wassermann result was indeterminate 23 times. None of the Vernes readings were such as to enable one to affirm syphilis. The serums that gave photometric readings above 10 (thirty-four in number) presumably represent a group that possess high flocculence normally.

DIAGNOSIS	NO.	PHOTOMETRIC READING
Alcoholism	1	13
Arteriosclerosis	1	15
Cephalalgia	1	10
	1	11
Epilepsy	1	13
	1	15
Facial neuralgia	1	14
Frontal sinusitis	1	18
Hemiplegia	1	17
Myasthenia	1	12
Myositis	1	12
Myxedema	1	11
Neuritis	1	13
(traumatic of ulnar)		
	1	19
	1	18
	1	16
Psychoneurosis	3	15
	3	13
	2	12
	4	10
Speech Defect	1	12
Sydenham's chorea	1	16
Taenia circinata	1	14

TABLE I

SHOWING COMPARATIVE WASSERMANN AND VERNES RESULTS ON 758 BLOOD SERUMS OF NONSYPHILITICS

NO. SERUMS	WASSERMANN RESULTS	VERNES RESULTS
701	Negative	0 to 9
31	Negative	10 to 20
3	Negative	21 to 24
23	Indeterminate	0 to 7
758		

II. Syphilitic Group

Five hundred and twenty serums were examined from cases of known syphilis. They were subdivided as follows:

1. Neurosyphilis	366
2. Congenital syphilis	14
3. Vascular syphilis	7
4. Latent syphilis	133
(Treated and untreated)	
	520

Most of these cases were under treatment at our clinic or had received treatment elsewhere. Repeated serologic examinations were made, in some instances ten or more; therefore, we were able to study the oscillations of the photometric results from graphs.

Space does not permit a detailed record of these results and a comprehensive idea of the comparison of the two tests cannot be shown in a condensed tabulation. A proper interpretation of the significance of Vernes results requires a knowledge of the amount and periodicity of treatment as well as the intervals between the successive tests. These features can be seen by a glance at the charts.

CASE 1.—J. A., admitted July, 1919, male, age 32. Syphilis contracted 1915. Treatment begun immediately.

Complaints.—Nervousness, irritability, precordial pain, weakness.
Neurologic Examination.—Negative.
Blood Wassermann: ++++.

NAME J.A.　　　　　　　No. 25890　　　　ETIOL Syphilis
　　　　　　　　　　　　　　　　DIAGNOSIS: ANAT. PATH Meningo Vaso.

	1922		1923								CLINIC	1924		
DATE	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Oct	Mar.	May	July
ARSPH	2	3	0	0	0	0	0	0	0	0	1 - 4 - 0		0	0
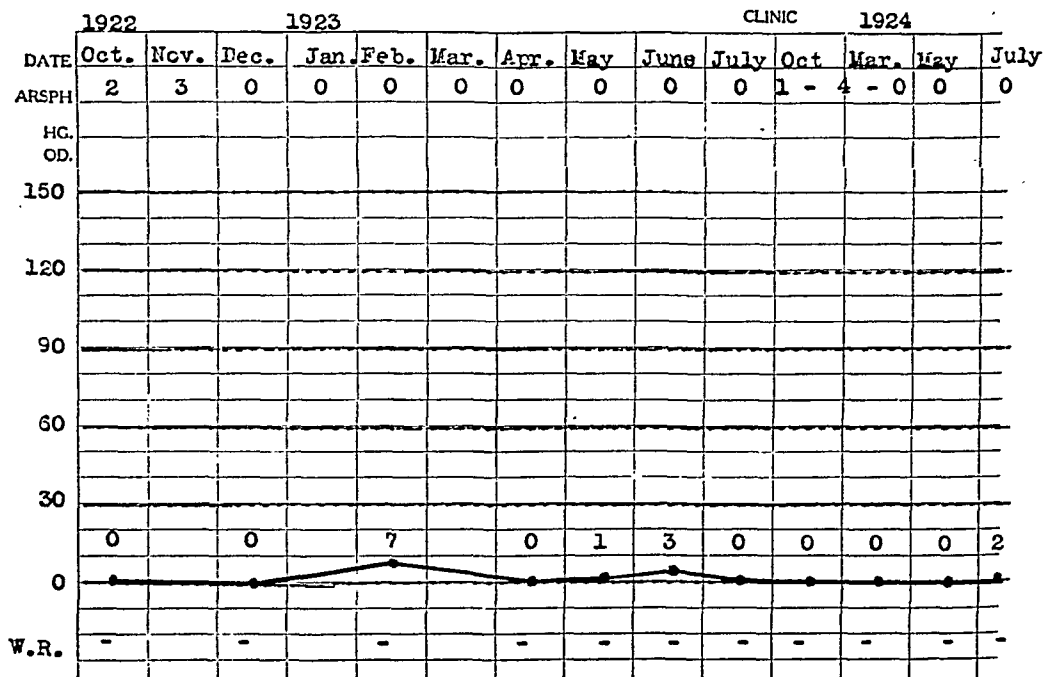
Chart I.

Spinal Fluid: Cells 6, globulin +, Wassermann ++++ in 0.2 c.c.; Lange 44332210000.
Diagnosis.—Meningovascular neurosyphilis.
Treatment July, 1919, to March, 1921: 28 salvarsan injections intravenously; 14 mercury injections intramuscularly, 9 intraspinal injections—salvarsanized serum. Spinal fluid became negative July, 1920. Blood fluid became negative March, 1921.

This case has shown a negative Wassermann reaction on eight occasions during a period of 17 months. The Vernes has shown an oscillation of only 7 points during this period. We believe that the spirochetosis has been eradicated. See Chart I.

CASE 2.—A. A., admitted July, 1919, female, age 25, wife of patient reported as Case 1. Positive Wassermann discovered 1917, after a miscarriage.
Complaints.—Loss of weight, weakness, dizziness, pains in legs.

Neurologic Examination.—Negative except for tremor of hands and inequality of patellar reflexes.

Blood Wassermann: ++++.

Spinal Fluid: Cells 0, globulin normal, Wassermann ++++ in 0.8 c.c.; Lange 1233210000.

Diagnosis.—Meningovascular neurosyphilis (conjugal).

Treatment August, 1919, to June, 1922: 24 salvarsan injections intravenously; 5 mercury injections intramuscularly; 7 intraspinal injections—salvarsanized serum.

Blood became negative in June, 1922. Spinal fluid became negative in all respects except Lange 4422231000 in June, 1920.

The Wassermann reaction between April, 1923, and May, 1924, varied from one-plus to doubtful. The Vernes curve shows an oscillation of greater ampli-

NAME   A.A.   No. 25891   ETIOL   Syphilis
                                        DIAGNOSIS. ANAT. PATH   Meningo Vasc.
                                                   CLINIC   1924

DATE	1922 Dec.	1923 Jan.		Apr.	May		July		Sept.	Oct.	Mar.	Apr.	May
ARSPH	0	0		0	0		0		0	6	0	0	0
HG. OD.													
150													
120													
90													
60													
30													20	
	0	6		8	10		5		10		0		1
0													
W.R.	-	-		?	+		?		-		?	?	?

Chart II.
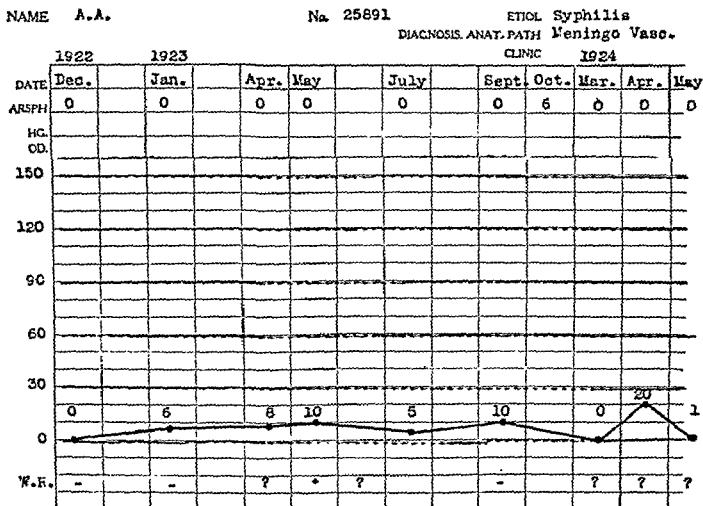
tude than one obtains with nonsyphilitic serum. The low points of 0 show that the normal flocculence in this case was not high. Note that in April, 1924, the curve rose to 20, whereas, the Wassermann remained unchanged. The single results would not be especially significant but the curve is illuminating. The infection is not controlled and further treatment is indicated. See Chart II.

CASE 3.—B., admitted November, 1920, female, age 54. Conjugal syphilitic infection, 1902. Husband a tabetic.

Complaints.—Insomnia, tinnitis aurium, anxiety, diplopia.

Neurologic Examination.—Left ptosis, slight ataxia, pupillary inequalities with Argyll Robertson on the right side, diminution of both corneal reflexes.

Blood Wassermann: +++.

Spinal Fluid: Cells 9, globulin trace, Wassermann ++ in 1.0 c.c.; Lange 4424332211.

Diagnosis.—Meningovascular neurosyphilis.

Treatment from November, 1920, to October, 1922: 65 salvarsan injections intravenously; 34 mercury injections intramusculary; 5 intraspinal injections—salvarsanized serum. Blood first became negative in June, 1921. Remained so until January, 1922, when it was +. Spinal fluid in April, 1921: Cells 3, globulin trace, Wassermann: Negative in 2.0 c.c.; Lange 5555543210.

In so far as one can judge clinically, the infection has been arrested in this case. It will be noted from Chart III that there is a considerable inconsistency in the Wassermann and Vernes results. In comment, I can say that with assurance that the Vernes results tally more accurately with the clinical aspects of the case than do the Wassermann results. See Chart III.

NAME B.   No. 829   ETIOL Syphilis
                                                    DIAGNOSIS: ANAT. PATH Meningo Vasc.

	1922			1923						1925 CLINIC			
DATE	Oct.	Nov.	Dec.	Jan.		Apr.		July		Mar.	Apr.	May	July
ARSPH	0	0	0	0	0	0	0	0	8	0	0	0	
HG. OD.	2	4	0	0	7	0	2	4	15	2	0	Hg.I.	gr.1/10
150													
120													
90													
60													
30													
0	6		3	2		6		1		0	0	0	0
W.R.	-		?	++		++++		++		++	++++	+	?
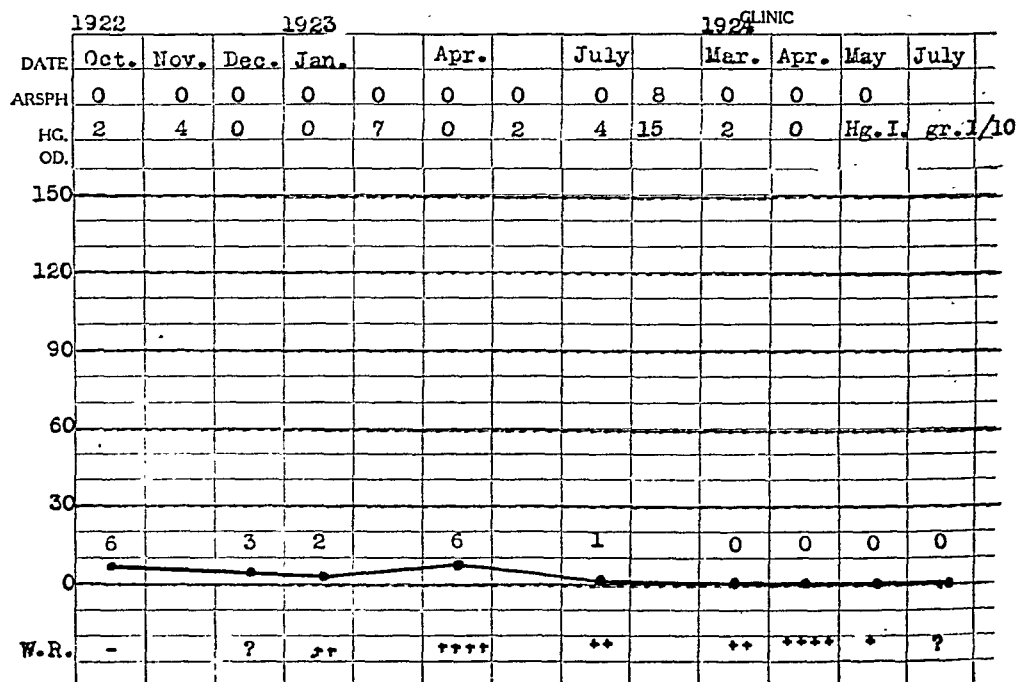
Chart III.

CASE 4.—B., admitted January, 1923, male, age 41. Syphilis admitted. Date of infection unknown.

Complaints.—Gastric crises, shooting pains in legs and shoulders.

Previous Treatment.—30 salvarsan injections intravenously; 40 mercury injections intramuscularly; 2 intraspinal injections—salvarsanized serum.

Neurologic Examination.—Slight swaying in Romberg, tremor of hands and tongue, Argyll Robertson pupils, right facial paresis.

Blood Wassermann: +++.

Spinal Fluid: Cells 0, globulin normal, Wassermann +++ in 1.0 c.c.; Lange 1111000000.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis, partial tabetic syndrome.

Treatment from January, 1923, to April, 1923: 7 salvarsan injections intravenously; 27 mercury injections intramuscularly; 3 intraspinal injections—salvarsanized serum. At the end of that time the blood was doubtful and the spinal fluid essentially unchanged.

Note the oscillation of seventeen points on the Vernes curve between April and July, during which time no salvarsan was administered. When the Vernes was highest the Wassermann was negative. See Chart IV.

CASE 5.—C. B., admitted January, 1920, male, age 41. Syphilis admitted. Date of infection unknown.

Complaints.—Painless swelling of left ankle (Charcot's joint) numbness of both feet, ankles, finger tips, and left leg, girdle sensation.

Neurologic Examination.—Tremor of fingers, ataxia, atrophy of left calf muscles, absent patellar reflexes, inequality of pupils with sluggish reaction to light on the left side.

Blood Wassermann: ++++.

Spinal Fluid: Cells 19, globulin ++, Wassermann ++++ in 0.5 c.c.; Lange 5554332100.

NAME B. No. 36430 ETIOL Syphilis
DIAGNOSIS: ANAT. PATH Mixed Form
CLINIC Early Tabes

1923											
DATE	Jan.	Feb.	Mar.	Apr.	May	July	Aug.				
ARSPH.	7	0	0	0	0	0	0				
HG.	2	1	5	4	5	3	4				
OD.											
150											
120											
90											
60											
30			23	32 2		18					
0				1		0					
W.R.				–	– ++						
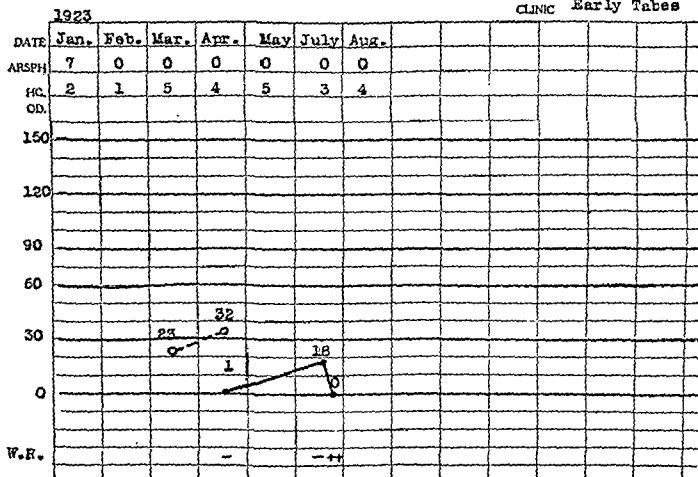
Chart IV.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis of encephalospinal distribution.

Treatment from January, 1920, to October, 1922: 40 salvarsan injections intravenously; 59 mercury injections intramuscularly; 22 intraspinal injections—salvarsanized serum.

This was a case of deeply seated neurosyphilis. The foci of infection were resistant to the action of spirocheticides probably on account of the failure of the drugs to penetrate. Although both reactions were positive in blood and spinal fluid, the Vernes indicates the changing character or intensity of the infection from time to time. It would appear that this feature of the Vernes test should be a valuable guide for the determination of the most efficient dosage and periodicity. A few doses of arsenicals properly spaced may be the means of arresting or overcoming an infection that otherwise would progress. In this

respect the conquest of syphilis might be compared to a hurdle race, which is only won after the last hurdle is cleared. If the runner should fail at the last obstacle the energy expended up to that point goes for naught and his race is lost. So in the treatment of syphilis, energetic treatment must be pursued till the last barrier is penetrated provided such a result is possible. If the Vernes curve is descending, treatment should be so adjusted as to favor the continuation of the descent to its base line. See Chart V.

NAME C.B.   No. 29217   ETIOL Syphilis

DIAGNOSIS: ANAT. PATH Mixed Form

CLINIC

DATE	1922		1923					1924						
	Oct.	Nov.		Apr.		Sept			Mar.		July			
ARSPH	1	1	4	3	4	0	4	7	0	0	0			
HG.	0	0	4	0	10	0	3	8	1	0	0			
OD.			206											

Chart V.
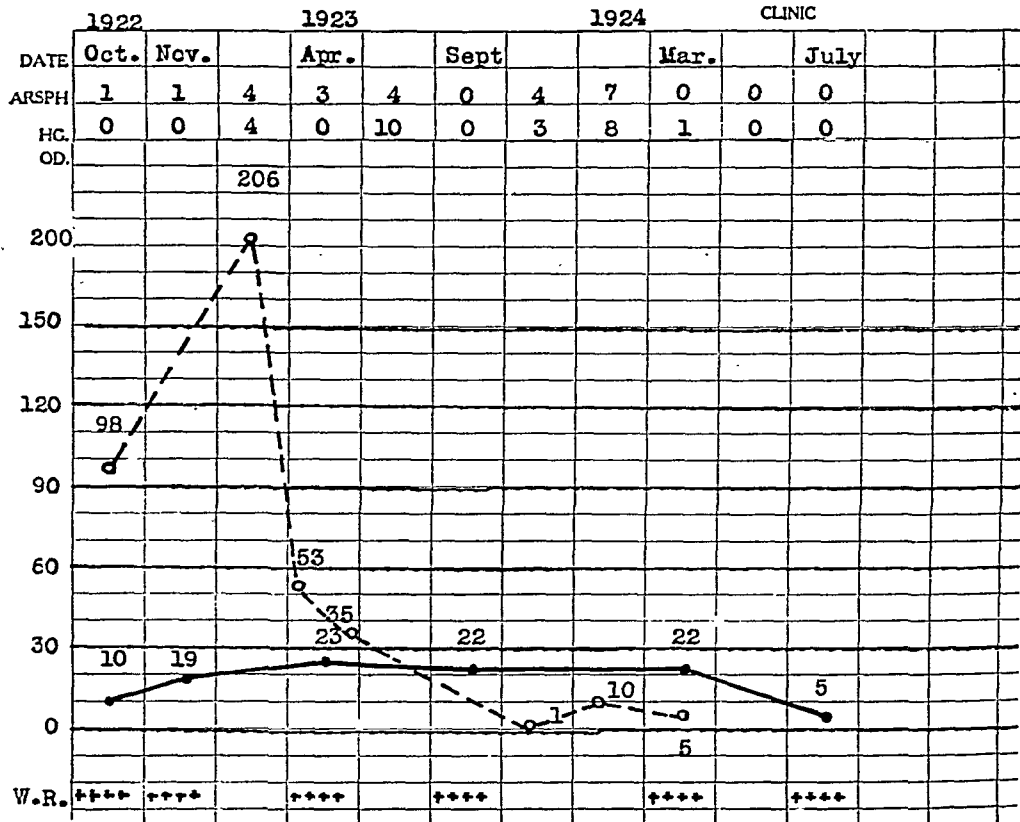
CASE 6.—C. L., admitted November, 1918, female, age 43. Probable conjugal infection. Duration of symptoms seven years.

Complaints.—Loss of weight, pain in back, and failing memory.

Neurologic Examination.—Romberg positive, absent patellar reflexes, absent right Achilles reflex, inequality of pupils and sluggishness of reaction to light.

Blood Wassermann: ++++.

Spinal Fluid: Cells 23, globulin ++++, Wessermann ++++ in 0.4 c.c.; Lange 1123210000.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis. Partial tabetic syndrome.

Treatment from November, 1918, to March, 1923: 14 salvarsan injections intravenously; 52 mercury injections intramuscularly; 9 intraspinal injections—salvarsanized serum. Blood Wassermann persistently ++++. Spinal fluid became negative in January, 1921.

Unfortunately this patient is intolerant to arsenic, reacting with severe dermatitis to small amounts. Therefore, mercury, bismuth, and the iodides have had to be employed. The resistant foci are probably in the walls of the blood vessels. See Chart VI.

CASE 7.—L. P., admitted October, 1918, male, age 41. Infection contracted in 1915.

Complaints.—Shooting pains in legs, bladder disturbances, failing vision, diplopia.

Neurologic Examination.—Positive Romberg, coarse regular tremor of both hands, inequality of deep reflexes, inequality of pupils, bilateral Argyll Robertson pupils.

Blood Wassermann: ++++.

Spinal Fluid: Cells 20, globulin ++, Wassermann ++++ in 0.2 c.c.; Lange 5555443000.

NAME C.L.   No. 23163   ETIOL Syph.

DIAGNOSIS. ANAT. PATH Mixed Form

CLINIC Early Tabes

DATE	Mar.	Apr.	May	June	July	Aug	Sept.	Oct.	Nov.	Dec.	Jan.			
ARSPH	0	0	0	0	0	0	0	0	0	0	0			
HG.	3	1	0	0	4	4	2	4	1	2	1			
OD.														
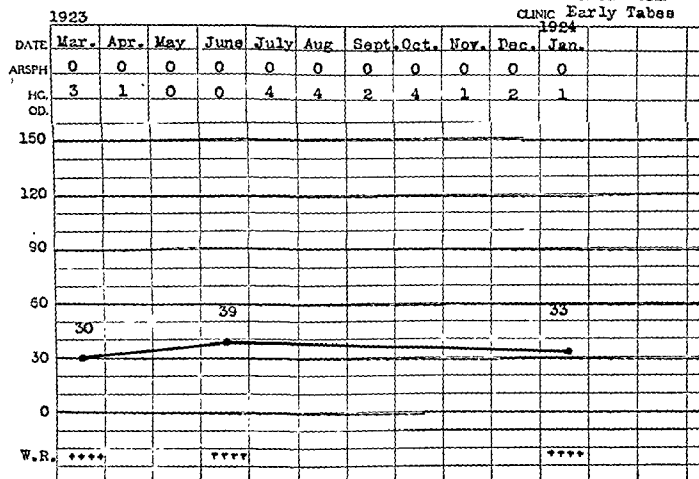
Chart VI

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis with syphilitic encephalitis.

Treatment October, 1918, to October, 1922: 67 salvarsan injections intravenously; 113 mercury injections intramuscularly; 38 intraspinal injections—salvarsanized serum.

After treatment.—Blood Wassermann persistently ++++, spinal fluid unchanged.

Treatment in this case has been pursued over a period of six years. The neural pathology is believed to involve meninges, blood vessels and parenchyma. An episode occurred, in 1922, typical, in its psychic and somatic manifestations, of general paresis. By persistent treatment with only short and widely separated periods of rest the economic efficiency of the patient has been preserved so as to enable him to successfully conduct a business that furnishes a livelihood to his family of five and a staff of ten or more employees. See Chart VII.

CASE 8.—G. W., admitted June 9, 1922, male, age 41. Syphilitic infection twenty years previous.

Complaints.—Pain in back and legs, unsteady gait.

Neurologic Examination.—Ataxia, thick and hesitating speech, coarse tremor of fingers, absent patellar reflexes, sluggish pupillary reaction to light.

Blood Wassermann: +++.

Spinal Fluid: Cells 25, globulin ++, Wassermann +++ in 1.0 c.c.; Lange 2333210000.

Diagnosis.—Ectodermal neurosyphilis, tabes dorsalis.

Treatment previous to October, 1922: 15 salvarsan injections intravenously; 15 mercury injections intramuscularly; 6 intraspinal injections—salvarsanized serum. Blood Wassermann became negative after 3 salvarsan injections. Spinal fluid became negative in May, 1923.

NAME **L.P.**   No. **22830**   ETIOL **Syphilis**

DIAGNOSIS: ANAT. PATH **Mixed Form**

CLINIC **Encephalitis**

	1922			1923						1924				
DATE	Oct.	Nov.		Mar.	Apr.	May				Mar.	Apr.	May	June	July
ARSPH	3	1		1	2	4	10	5	4	3	1	3	1	
HG. OD.	0	0		0	2	3	15	10	3	4	0	2	2	

Chart values (Vernes curve): 1, 10, 11, 7, 26, 14

W.R.: − , ꓔꓔꓔꓔ , +++ , ++ , ꓔꓔꓔꓔ , +++
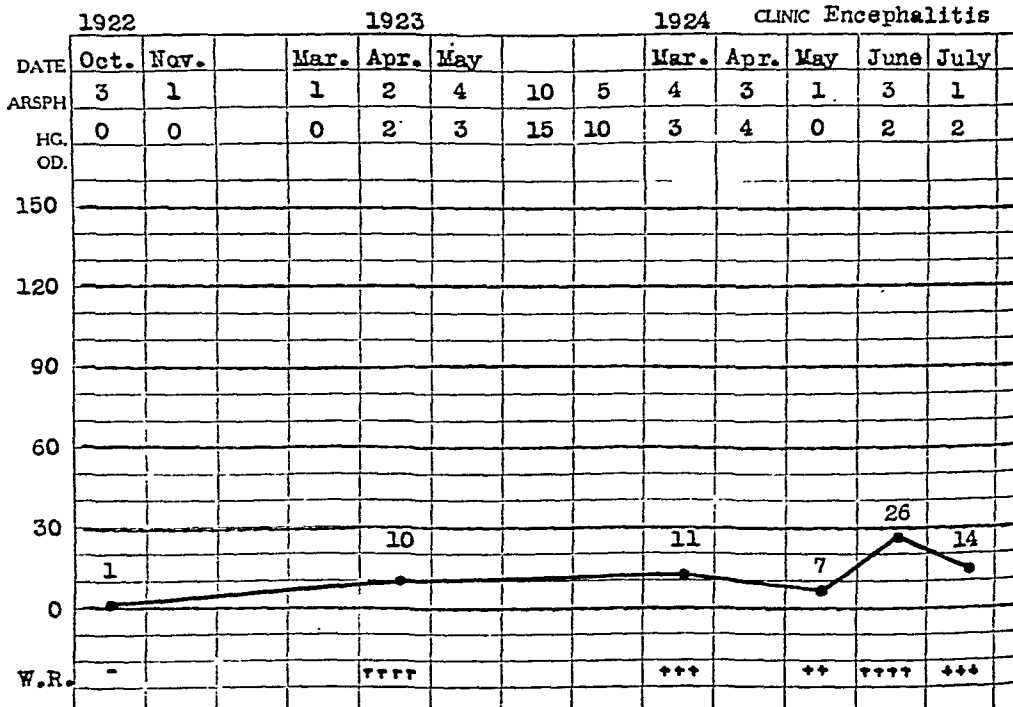
Chart VII.

This case exemplifies the clinical arrest of a tabetic syndrome that began with severe lightning pains and locomotor difficulty. The Vernes curve ascended 13 points while the Wassermann remained negative or doubtful. See Chart VIII.

CASE 9.—T. H., admitted March, 1923, male, age 37. Syphilis denied.

Complaints.—Insomnia, lack of ambition, lack of ability to concentrate.

Neurologic Examination.—Tremor of hands, hyperactive deep reflexes.

Blood Wassermann: ++++.

Spinal Fluid: Cells 0, globulin negative, Wassermann negative; Lange 1111100000.

Diagnosis.—Vascular type of mesodermal neurosyphilis with anxiety psychoneurosis.

An illustration is here shown of the necessity of successive Vernes reactions in order to establish a curve. The results considered individually have

but little significance, but the oscillation (descent) of 14 points during four months of treatment and the subsequent ascent of 10 points during which period the Wassermann remained practically stationary illustrates the comparative significance of the two tests. (Chart IX.)

CASE 10.—A. J., admitted May, 1923, male, age 50. Syphilitic infection denied.
Complaints.—Insomnia, precordial pain, palpitation of the heart, anxiety.
Neurologic Examination.—Fine tremor of both hands, thick speech, otherwise negative.
Blood Wassermann: +++.
Spinal Fluid: Cells 8, globulin +, Wassermann ++++ in 0.2 c.c.; Lange 1122210000.
Diagnosis.—Meningovascular neurosyphilis with anxiety psychoneurosis.

NAME G. W.   No. 990   ETIOL Syphilis
                                                      DIAGNOSIS ANAT. PATH Ectodermal
                                                              CLINIC Tabes Dorsalis

DATE	1922		1923							1924		
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May		Feb.	Mar.	Apr.
ARSPH	4	4	1	4	4	1	4	4	8	0	4	4
HG. OD.										6	6	
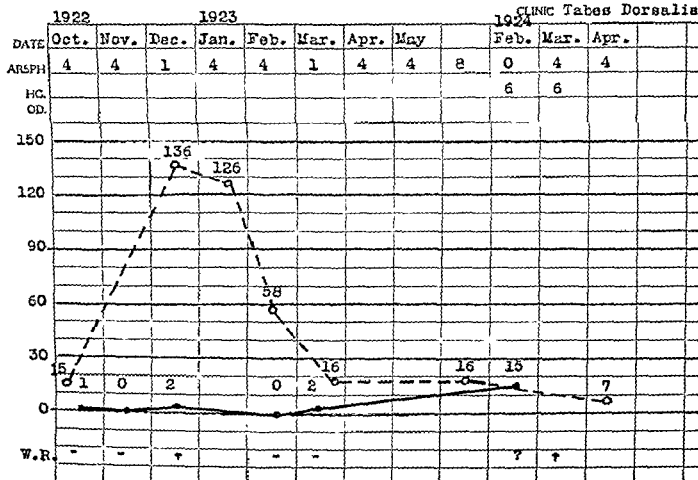
Chart VIII.

The comparative flexibility of the two tests may be seen. While the Wassermann remained four-plus the Vernes oscillated between 0 and 40. After reaching 0 the Vernes maintained this base line but the Wassermann fluctuated. See Chart X.

CASE 11.—F. R., admitted May, 1919, male, age 53. Syphilitic infection denied.
Complaints.—Pains in shoulders and back, girdle sensation.
Neurologic Examination.—Negative except for hyperactive patellar reflexes.
Blood Wassermann: ++++.
Spinal Fluid: Cells 4, globulin +, Wassermann negative in 2.0 c.c.; Lange 0000000000.
Diagnosis.—Vascular neurosyphilis, syphilitic aortitis.
Treatment from May, 1919, to October, 1922: 23 salvarsan injections intravenously; 58 mercury injections intramuscularly.

This case has been considered Wassermann fast. The Vernes oscillation confirms the Wassermann but its low character suggests that properly spaced treatment would be effective in permanently obliterating this oscillation. In such a case with divergent results of these two serologic tests, clinical facts must determine the relative value of the two findings. In this particular case the medical features consisted of myocarditis, aortitis, and the vascular type of kidney disease. See Chart XI.

NAME T.H.   No. 36859   ETIOL Syph.
DIAGNOSIS: ANAT. PATH Vascular
CLINIC Psychoneurosis

1923

DATE	Mar.	Apr.	May	June	July	Aug	Sept.	Oct.	Nov.	Dec.			
ARSPH	0	4	4	0	0	0	0	3	5	0			
HG. OD.	0	4	4	5	2	2	3	0	0	0			
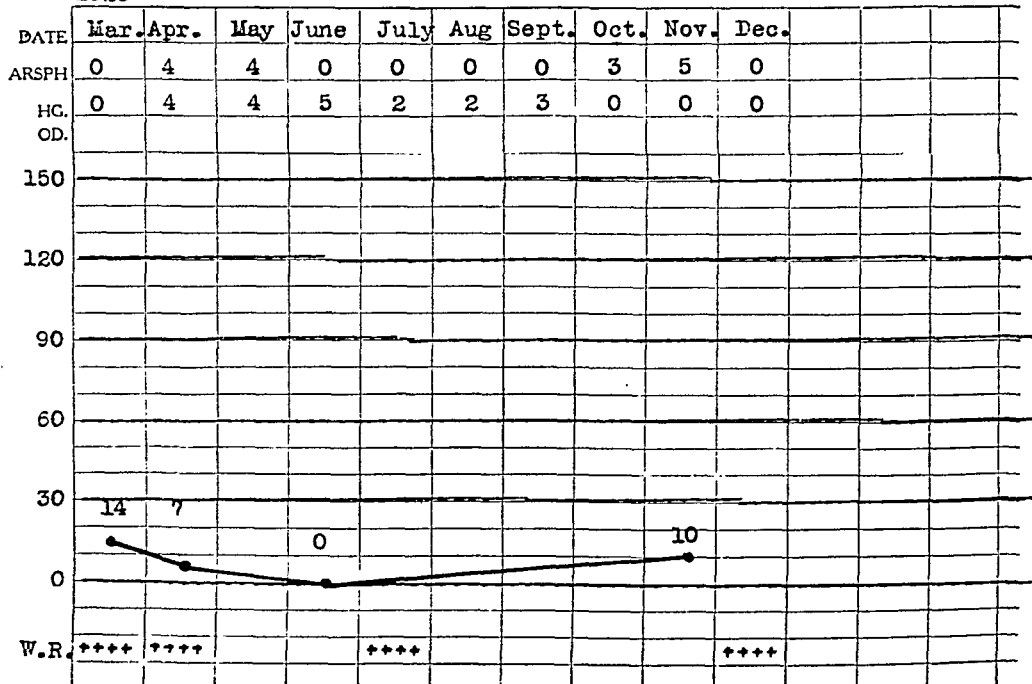
Chart IX.

MISCELLANEOUS GROUP

Nineteen serums were included in this group comprising cases concerning which there was reasonable uncertainty. The histories and clinical examinations did not warrant placing them in any of the other groups. Some denied syphilis but had positive Wassermann reactions or hyperflocculence, others said that they had had syphilis but it was not confirmed clinically or serologically. The whole group requires further study.

Three cases were diagnosed clinically as possible neurosyphilis. There was no history of infection. Wassermann reactions were negative and the Vernes results were 5, 3, and 0.

Five other serums were negative to the Wassermann reaction but four of these gave Vernes results requiring further study before a definite opinion was warranted and the fifth gave a Vernes result of 76 definitely affirming syphilis.

One case admitted syphilis but clinically presented only psychoneurotic symptoms. The Wassermann was four-plus and the Vernes 7.

Two cases were the wives of syphilitics whose serology was done as a precautionary measure. They presented no symptoms. The Vernes results were 8 and 69 and the Wassermann reactions of both were four-plus.

There were two cases of general arteriosclerosis and hypertension with no history of syphilis. In one, the Wassermann was two-plus and Vernes 0; in the other, the Wassermann was four-plus and the Vernes 0.

One case with right facial paresis admitted syphilis. The Wassermann was four-plus and the Vernes 1.

One case of myocarditis denied syphilis but had a four-plus Wassermann and Vernes of 66.

NAME A.J.   No. 37167   ETIOL Syph.
                                        DIAGNOSIS. ANAT. PATH Meningo Vasc.
                                                  CLINIC Psychoneurosis

1923						1924						
DATE	May	June	Sept.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
ARSPH	0	2	6	0	2	1	1	0	1	1	2	1	2	0
HG. OD.	0	0	2	3	5	4	4	2	0	0	4	4	5	2

Chart X

Four cases that denied syphilis had positive Wassermann serology and Vernes results of 7, 5, 13, and 5 respectively.

From a clinical standpoint, ten of the cases included in this group were considered as probably syphilitic (1, 2, 3, 5, 7, 8, 9, 10, 11, 14). The Wassermann results supported this conclusion in four cases (9, 10, 11, 14). Single Vernes results were confirmatory of syphilis in two cases (5 and 11) and in four cases (7, 8, 9, and 10) were insufficient to affirm or deny syphilis without further investigation.

In eight cases (4, 6, 12, 13, 16, 17, 18, 19) syphilitic infection was denied and clinically they were regarded as nonsyphilitic. The Wassermann was negative in two (4 and 6), four-plus in two (13 and 19), two-plus in three (12, 17, 18) and one-plus in one (16). Single Vernes results indicated probable

syphilis in two cases (4 and 6) in which the Wassermann was negative and suggested the necessity of further investigation in one case (18) in which the Wassermann was two-plus. The other five Vernes results, taken alone, signified only that there was no hyperflocculence at the moment of examination.

One case of myocarditis (15) in which syphilis was denied gave Wassermann and Vernes results confirmatory of syphilis—four-plus and 66.

<div align="center">SPINAL FLUIDS</div>

Up to October, 1923, one hundred and sixty-nine spinal fluids from sixty-two cases of neurosyphilis were submitted to both Wassermann and Vernes

NAME F.R.   No. 25125   ETIOL Syphilis
                                      DIAGNOSIS: ANAT. PATH Vascular
                                      CLINIC Aortitis

	1922			1923								
DATE	Oct.			Apr	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
ARSPH	0	0	0	0	0	0	0	0	0	0	0	0
HG. OD.	2	4	1	0	4	2	0	2	0	0	0	0
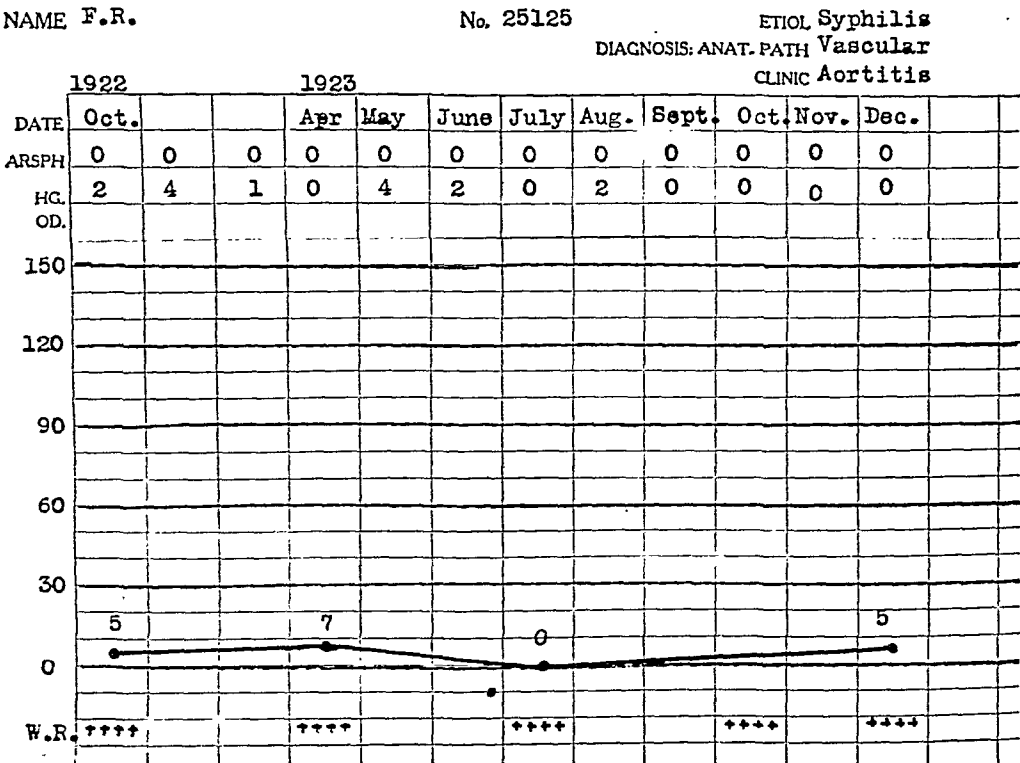
Chart XI.

tests. None of the results of the fluid examinations from nonsyphilitic cases or from any cases since the date mentioned have been submitted to a critical analysis.

For clinical purposes the cases were classified as follows:

	Fluids	Cases
Mesodermal (meningovascular) neurosyphilis	57	25
Ectodermal (parenchymatous) neurosyphilis	112	37
	169	62

The technic was that originally recommended by Vernes. A result above 50 warrants the unqualified diagnosis of neurosyphilis. Results between 30 and 50 justify a strong presumption of neurosyphilis but require more than

TABLE II

SHOWING THE SEROLOGIC RESULTS AND THE CLINICAL DIAGNOSES IN 19 BLOOD SERUMS FROM A GROUP OF CASES IN WHICH THE DIAGNOSIS OF SYPHILIS COULD NOT BE AFFIRMED CLINICALLY

NO.	W. R.	V. R.	DIAGNOSIS AND REMARKS
1.	–	5	Neurosyphilis
2.	–	3	Neurosyphilis
3.	–	0	Neurosyphilis—Rt. hemiparesis
4.	–	29	Vertigo—Syphilis denied
5.	–	76	Hemiparesis of sudden onset—Syphilis denied
6.	–	24	Psychoneurosis—Syphilis denied
7.	–	19	Wife of a paretic. No symptoms of neurosyphilis
8.	–	15	Psychoneurosis. Affirms syphilitic infection in 1918
9.	+ + + +	7	Psychoneurosis. Syphilis admitted
10.	+ + + +	8	Wife of a syphilitic—No symptoms
11.	+ + + +	69	Wife of a syphilitic—No symptoms
12.	+	0	Arteriosclerosis and hypertension—Syphilis denied
13.	+ + + +	0	Arteriosclerosis and hypertension—Syphilis denied
14.	+ + + +	1	Right facial paresis—Syphilis admitted
15.	+ + + +	66	Myocarditis—Syphilis denied
16.	+	7	Paranoid trend—Syphilis denied
17.	+ +	5	Psychoneurosis—Syphilis denied
18.	+ +	13	Epilepsy—Syphilis denied
19.	+ + + +	5	Psychoneurosis—Syphilis denied

one examination in order to establish two points for the graph. The oscillation then determines the interpretation.

To utilize the analogy previously mentioned, we may repeat that as the normal temperature is subject to individual variation so is the normal flocculence of the spinal fluid. Zero may be the normal flocculence for some individuals, 10 for others and 30 for others. Normal flocculence does not exceed 50 and oscillation of more than a few points from one time to another does not occur in nonsyphilitic fluids.

On the basis of single fluid examinations the Wassermann and Vernes reactions agreed 113 times and disagreed 56 times. Of the fifty-six disagreements the Wassermann was positive and the Vernes less than 30 in 52 cases, the Wassermann was negative and Vernes more than 30 in 4.

The 56 fluids that showed a variance between the two tests in individual examinations came from 29 cases. Fifty-one other fluid examinations from these same cases were in agreement.

Inasmuch as the interpretation of this reaction depends upon a study of the curve that is obtained by plotting the results of successive examinations, we can only gain a proper idea of its value as compared with other tests by studying these curves.

An analysis of the 29 cases in which instances of variation occurred in certain single examinations disclosed the following facts:

Ten cases had but one spinal fluid examination. The Wassermann was negative and the Vernes greater than 30 in 1 case. The Wassermann was positive and the Vernes less than 30, nine times.

Three cases had two fluid examinations each.   In two of these the Vernes curve agreed with Wassermann results and in the third, a case of treated tabes dorsalis, the Wassermann was positive and the Vernes less than 30.

Sixteen cases had from 3 to 7 fluid examinations each.   The Vernes curves agreed with the Wassermann results in fifteen and in the sixteenth the Wassermann was negative and the Vernes curve above 30.   In other words the Vernes curves indicated syphilis in all of these sixteen, the Wassermann was positive in fifteen.   The case in which the Wassermann and Vernes disagreed was one of treated tabes dorsalis.   The Wassermann was negative on three examinations but the Vernes showed hyperflocculence twice and normal once.

These fluid results on cases of confirmed neurosyphilis may be summarized by saying that:

1. Where three or more examinations were made, the Vernes curves indicated the activity of syphilis in sixteen cases and the Wassermann was positive in fifteen.

2. In three cases where only two examinations were made the Wassermann was positive in all and the Vernes curves gave a presumption of syphilis in two.

3. When the results of single examinations were compared (10 cases), the Wassermann was positive and the Vernes less than 30 in nine, the Wassermann was negative and the Vernes more than 30 in one.

The Vernes reaction is a valuable addition to the serologic procedures for the diagnosis of syphilis.

It is especially useful for the determination of the effect of therapy.

Successive results illustrate graphically whether the infection is progressing, remaining stationary, or regressing.

REFERENCES

[1]Cornwall, L. H., and Aronson, L. S.:   Jour. Am. Med. Assn., Dec. 18, 1920, lxxv, 1697-1701.
[2]Cornwall, L. H.: Arch, Dermat. and Syph., April, 1922, v, 433-461.
[3]Vernes, A.:   Compt. rend Acad. d. sc., Nov. 26, 1917, clxv, 22; Presse méd., Dec. 13, 1917, xxv, 69; Compt. rend Acad. d. sc., April 8, 1918, clxvi, 14; ibid., Sept. 2, 1918, clxvii, 10; ibid., Sept. 30, 1918, 14; ibid., Dec. 9, 1918, 24; ibid., Jan. 27, 1919, clxviii, 4.
[4]Sachs, H., and Georgi, W.:   Med. Klin., August 18, 1918, xiv, 805.
[5]Kahn, R. L.:   Arch. Dermat. and Syph., May 1922, v, 570; June, 1922, v, 734.
[6]Vernes, A.:   Presse méd., Dec. 3, 1921, xxix, 97.
[7]Vernes, A.:   Travaux et Publications de l'Institute Prophylactique, Part II, Paris, 1923, A. Maloine et Fils.
[8]Williams, H. B.:   Jour. Biol. Chem., Sept. 3, 1912, 317-347.

LABORATORY METHODS

SIMPLE APPARATUS FOR CORONARY PERFUSION OF THE MAMMALIAN HEART[*]

By W. T. Dawson, B.A., (Oxon.), M.A., Philadelphia, Pa.

THIS apparatus is a modification of that of Gunn.[1] The principal difference lies in a more convenient arrangement for heating the perfusion fluid, by transfer of heat from a Bunsen flame by conduction along a metal rod to the water of the outer jacket, setting up convection currents in the water there, resulting in even heating of the perfusion fluid flowing along the inner tube. Such an arrangement appears to have been first used by Smith[2] and was last year independently worked out by myself, and is here reported, with the consent and by the advice of Dr. Smith, in detail, in the hope that such a description may be of value to teachers.

DESCRIPTION OF THE APPARATUS

Two Ringer-Locke reservoirs are provided, in which the Ringer is at room temperature. They may conveniently be placed 20 to 50 cm. above the top of the inner tube. They are connected through a small Y-tube with the inner tube of the perfusion apparatus. The upper end of the inner tube is furnished with a one-hole stopper; the tube may be of thin glass to facilitate conduction of heat but I have not found this necessary; as to diameter, it is selected of such a size as just to admit a "twelve-inch chemical" thermometer; the thermometer rests on a constriction in the wall made by denting in the latter while hot and it is thus prevented from slipping down; the constriction is about 2 cm. above a cannula end which, for the rabbit heart, is about 5 mm. outside diameter; for cats a larger and for guinea pigs and rats a smaller cannula is rather desirable. The inner tube may be cut off below the constriction so that such cannulas may be inserted in the inner tube as in Gunn's apparatus; the space between the stopper at the top and the upper end of the thermometer is filled with glass beads. The inner tube is about 38 cm. long. The outer jacket, which contains water, is about 30 cm. long and 4 cm. in diameter, provided with two side tubes, one, bulbed at its junction, for the entrance of a piece of copper wire of about 6 mm. diameter (No. 4 gauge) and 20 cm. long; the other fitted with rubber tubing and pinchcock so as to facilitate letting all or part of the warm water out of the outer jacket in order to cause a more rapid fall of temperature of the perfusion fluid in studying the effect of temperature upon rate. The size of the rod limits the amount of heat conducted into the outer jacket per unit of time;

larger sizes, such as No. 3 or No. 2 may be necessary in perfusions of hearts of larger animals such as the dog; if they can be obtained they should, therefore, be used in making up the apparatus. The connection with the side-tube of the outer jacket is made by a sleeve of rubber tubing wired in place with soft copper wire; this connection does not seem to perish very quickly, and is cheap and easily renewed. A lighted Bunsen is set under the rod a few minutes before it is desired to use the apparatus, and while the perfusion is proceeding the temperature may be readily regulated by shifting
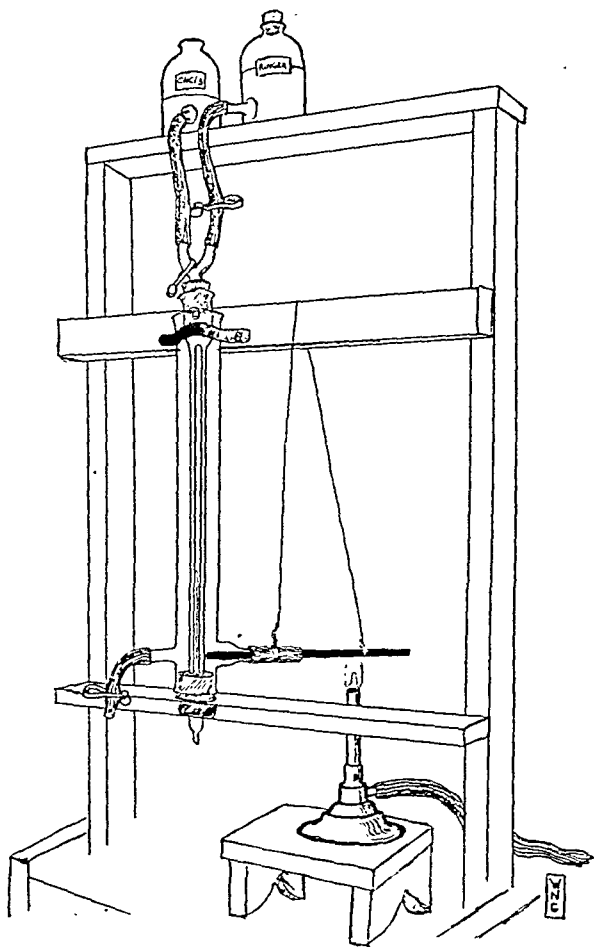
Fig. 1.

the burner to a suitable position to keep the temperature of the perfusion fluid constant. A short length of narrow rubber tubing, not shown in the diagram, is kept on the cannula end as a guard tube when the apparatus is not in use and also to aid in filling the inner tube. When this is desired the guard tube is closed off with a pinchcock, the rubber stopper at the upper end loosened and Ringer allowed to flow in from one of the reservoirs. When the inner tube is filled the stopper is pushed down into position, and a little Ringer run down through the inner tube by releasing the pinchcock on the guard tube. If both Ringer reservoirs be now clamped off, the Ringer will not escape from the inner tube when the guard tube is removed from the

cannula end to allow the heart to be tied on. The reservoirs and the inner tube should be drained of Ringer at the end of each day's work and rinsed with distilled water.

THE PERFUSION FLUID

The Ringer-Locke used has the following composition: NaCl, 0.92 per cent; KCl, 0.042 per cent; $CaCl_2$, anhydrous, 0.012 per cent; $NaHCO_3$, 0.015 per cent; several liters of this are made up in a large bottle and oxygenated by the passage through it of a stream of bubbles of oxygen for about half an hour; if well stoppered it keeps its oxygen content apparently sufficiently high for several days; just before placing it in the reservoirs one gram of glucose is added to each liter which dissolves rapidly. The formula given above agrees in calcium content with those given in the laboratory manuals of Sollman[3] and Jackson,[4] and others, assuming that "calcium chloride crystals" means $CaCl_2.6H_2O$. There are, however, several other crystalline forms. *The United States Pharmacopeia*, Edition 9, requires for general medicinal uses a calcium chloride which apparently must have the formula $CaCl_2.2H_2O$ or contain still less water than corresponds to this formula. Such a calcium chloride is, therefore, on the market. The difficulty of assigning any clear meaning to the description "calcium chloride crystals" may be easily seen from the following quotation from J. W. Mellor's *Modern Inorganic Chemistry*: "At 29.8° the hexahydrate $CaCl_2.6H_2O$ changes into the $\propto$-tetrahydrate $\propto$-$CaCl_2.4H_2O$; at 45.3° the $\propto$-tetrahydrate passes into the dihydrate $CaCl_2.2H_2O$; at 175.5° the dihydrate passes into the monohydrate $CaCl_2.H_2O$; and at 260° the monohydrate passes into the anhydrous salt $CaCl_2$." If it be true, as is very generally taught, that a delicate balance of inorganic positive ions is of supreme importance in physiologic salts solutions, the exact sort of calcium chloride used should be more exactly specified in textbooks and other publications than is now usually the case. Calcium chloride exists in at least five different forms; and each of these at ordinary temperatures and humidities is avid of water and, therefore, liable to change of calcium percentage.

PREPARATION OF THE HEART

The success of a perfusion depends largely on the condition of the heart before removal from the animal, so that it is desirable, if possible, to use hearts from freshly-killed unanesthetized animals. With bleeding, moreover, there appears to be some danger of air embolism of the coronaries. Speed is also an essential in getting the heart out of the rabbit. The animal is seized by the small of the back with the left hand; and while it hangs head down it is struck with the side of the right hand a sudden hard blow on the occiput so as to stun it completely. An assistant grasps the legs and stretches the rabbit out upon a table. With a pair of stout scissors the skin is split up the middle on the underside, and an opening at once made into the thoracic cavity by cutting through the ribs from diaphragm to neck on the right of the sternum; while this cut is being made the animal is turned on its left side so that as the lungs collapse the heart will fall away from the points of the scissors. The fingers of the two hands are now passed around

the chest of the rabbit just under the axillae, while the thumbs enter the opened thorax under the ribs at the borders of the cleft made into it. The thumbs are forced apart so as to dislocate the costal walls, which are pushed far back till the heart lies in easy sight and reach. The pericardium is picked up between thumb and forefinger, and split well open with small scissors. The heart is popped out by gentle manipulation around it, the tips of the fingers placed under it, the great vascular trunks lightly grasped with thumb and forefinger, and the heart, so protected from injury, liberated, by a semi-circular cut about two centimeters from its base, from the rest of the body. The mass is thrown into a beaker of Ringer, and when this becomes much stained with blood, into a second, and so on. The heart is prepared for tying onto the perfusion cannula by now trimming off the mass cut away with it, the aorta being identified as a white stout elastic tube and cut short before the origin of the innominate artery.

With small forceps the aorta is pinched off 2 mm. below the top, its lumen filled with Ringer from a medicine dropper, and the great artery pushed up over the cannula end of the inner tube, and tied in position. The beat may start up in such a fresh heart on perfusion at once; in fact, it usually continues beating during its passage through the beakers of Ringer at room temperature; as the perfusion fluid is warmed up the rate increases (see the beautiful records of effect of temperature changes in Sherrington,[5] pp. 11, 12). The optimum temperature is about 35° to 37° C. (Dixon,[6] p. 40). The heart may be started off, if the beat is not satisfactory, by injecting with a hypodermic syringe into the rubber exit tube of the Ringer reservoir in use 1 c.c. of 1:50,000 epinephrin or of 1:5,000 epinine.

RESISTANCE OF THE HEART TO LACK OF OXYGEN

If the room temperature be 20° C. or over the heart usually continues beating in the beakers of Ringer, and is then exposed to lack of oxygen. To this it is not particularly sensitive, apparently. It has recently been found that clamping off the circulation through the heart of the cat for eight minutes did not prevent the resumption at the end of that time of vigorous, normal contraction[7]; the much more sensitive cerebral cells were damaged beyond repair by three minutes cessation of the circulation. Gunn says,[1] moreover, that excised hearts may be successfully perfused after a period of several hours lying in Ringer at room temperature. This is possible probably where the temperature is so low that the hearts practically or altogether cease to beat. I was unable to confirm it with two guinea pig hearts kept for six hours at about 20° C.; the beat was revived but was not at any time good, though improved by injection into the rubber connection tube of adrenalin and 1 per cent CaCl$_2$ solutions. Starling and Verney apparently consider that, like skeletal muscle, cardiac muscle needs oxygen only for the recovery process, the actual contraction being anerobic.[8] This appears to be confirmed by the results of Hilton and Eichholtz.[9]

My thanks are due to Prof. H. C. Bazett, of the Medical Department of the University of Pennsylvania, for his advice and for giving me the facilities for having the apparatus made up; to J. H. Graham, the university glass-

blower, for making two models; and to William N. Goodell for kindly making the drawing.

SUMMARY

A modification of Gunn's apparatus for the perfusion of the coronary vessels of the mammalian heart is described, dimensions given, and some remarks made as to its use.

REFERENCES

[1]Gunn, J. A.: An Apparatus for Perfusing the Mammalian Heart, Jour. Physiol., 1913, xlvi, 506.

[2]Smith, M. I.: The Action of Strychnine in Certain Types of Cardiac Irregularities, Jour. Pharmacol. and Exper. Therap., 1917, ix, 365.

[3]Sollman, T.: A Laboratory Guide in Pharmacology, Philadelphia, 1917, W. B. Saunders Company.

[4]Jackson, D. E.: Experimental Pharmacology, St. Louis, 1917, C. V. Mosby Co.

[5]Sherrington, Sir Charles: Mammalian Physiology, A Course of Practical Exercises, 1919, Clarendon Press.

[6]Dixon, W. E.: Practical Pharmacology, New York, 1920, Macmillan Co.

[7]Cutler, E. C., Levine, S. A., and Beck, C. S.: The Surgical Treatment of Mitral Stenosis, Experimental and Clinical Studies, Arch. Surg., 1924, ix, 689.

[8]Starling, E. H., and Verney, E. B.: The Secretion of Urine as Studied on the Isolated Kidney, Proc. Roy. Soc., B, 1925, xcvii, 321.

[9]Hilton, R., and Eichholtz, F.: The Influence of Chemical Factors on the Coronary Circulation, Jour. Physiol., 1925, lix, 413.

AN IMPROVED APPARATUS FOR DETERMINATION OF CHOLESTEROL*

By S. L. Leiboff, Ph.B., New York.

DURING the last decade a great deal of work has been done on the cholesterol content of the blood during health and disease and valuable information was thus obtained. We now know that increased amounts are found in chronic and acute nephritis, diabetes, toxemias of pregnancy, cholelithiasis and lipemia. A marked decrease is found in pernicious anemia.

For this knowledge we are greatly indebted to Grigaut[1] who was the first to describe a colorimetric method for determining cholesterol in small amounts of blood.

At present there are three methods in use which give fairly good results. They are the following:

1. Digitonin gravimetric method of Windaus,[2] by which the cholesterol is precipitated as a compound of digitonin, and weighed. Only free cholesterol is precipitated by the digitonin. For the estimation of the total cholesterol, the cholesterol esters must first be saponified.

2. Bloor's[3] modification of the Autenrieth and Funk[4] method. This is based on the Liebermann-Burchard test, and is carried out as follows: 3 c.c. of blood are extracted with a mixture of alcohol and ether and the extract is

evaporated to dryness. The dry residue is then extracted with chloroform to which is added acetic anhydride and concentrated sulphuric acid, and left in a dark place until the color develops. It is then compared in a colorimeter with a standard containing a known amount of cholesterol.

3. Myers and Wardell method[5] which, like the former, is based on the Liebermann-Burchard test, but requires smaller amounts of blood. In this method 1 c.c. of blood is intimately mixed with plaster of Paris and dried in an electric oven. The dried plaster of Paris is then transferred to a paper extraction shell and is extracted with chloroform under a reflux condenser. Acetic anhydride and concentrated sulphuric acid are then added, and, when the color is developed, compared in a colorimeter.

Of the three methods just described, the digitonin method is the most accurate. However, it is not suitable for clinical work because it is extremely laborious and requires much skilful manipulation.
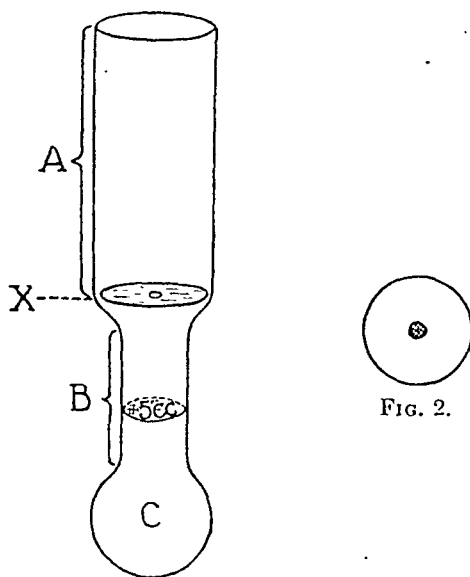
FIG. 1.

FIG. 2.

Bloor's method gives somewhat higher results than the Myers-Wardell method and is considered more accurate. However, it is very tedious and requires particular attention while the alcohol-ether extract is being dried, since the slightest overheating produces a brownish substance which greatly interferes with the color formation.

Up to the present the Myers-Wardell method has been the most suitable for clinical purposes, and the most widely used. This method, while the best at our disposal, has the following disadvantages: first, it is very time-consuming, and second, it is supposed to give somewhat low figures. The latter is apparently due to slight losses incurred while removing the dried plaster of Paris to the extraction shell, and while transferring the chloroform extract from the comparatively large flask to the small test tube.

Recently, I have described a simplified method for cholesterol determination[6] which gives somewhat more accurate results and saves a great deal of

time and labor. It is carried out as follows: About 5 c.c. of chloroform are put into the extraction tube (Fig. 1) and the filter paper disc (Fig. 2) is dropped into the extraction tube; it should lie at point X. The constricted portion of the tube, B, thus serves a double purpose: it holds the paper disc in place, and permits more accurate dilution.

Twenty-five hundredths c.c. of oxalated blood is pipetted on the filter paper disc. The blood is immediately absorbed by the paper and no drying
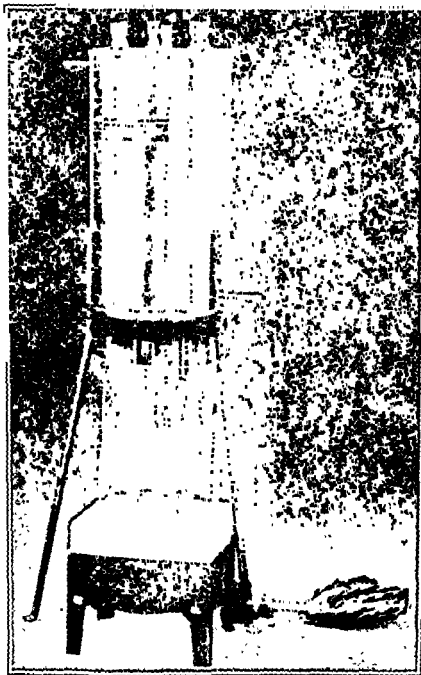
Fig. 3.

is necessary. The extraction tube is then attached to a reflux condenser and immersed in a beaker of hot or boiling water placed over a small electric stove. The water in the beaker should be above the level of the chloroform in the tube.

Extract for thirty minutes. Detach the tube from the condenser, remove the paper disc from the tube, and cool by immersing in cold water for a minute. When cool, add chloroform exactly to the 5 c.c. mark.

In a similar extraction tube place 5 c.c. of a standard solution containing

0.4 mg. of cholesterol per 5 c.c. This standard is prepared by dissolving 40 mg. of pure dry cholesterol in 500 c.c. of pure dry chloroform.

Add 2 c.c. of pure dry acetic anhydride and 0.1 c.c. of concentrated sulphuric acid to each tube. Insert cork stoppers into the tubes and invert twice to mix well. Place the tubes in a beaker of cold water for half a minute to cool, and leave in a dark place for ten minutes and read in colorimeter.

The standard is set in the colorimeter at 10 or 15 mm., depending on the intensity of the color of the unknown. The results are calculated by the following simple formula:

$$\frac{S}{R} \times 160 = \text{mg. per 100 c.c. of blood.}$$

S = reading of standard.
R = reading of unknown.

This method was checked up by the Myers-Wardell method. Cholesterol determinations were done by the two methods on blood specimens from dif-

TABLE I

SAMPLE NO.	M. AND W. METHOD MG. PER 100 C.C.	NEW METHOD MG. PER 100 C.C.	DIFFERENCE
1	165	174	+ 9
2	127	135	+ 6
3	220	233	+13
4	153	150	− 3
5	148	153	+ 5
6	139	146	+ 7
7	245	260	+15
8	129	134	+ 5
9	174	179	+ 5
10	140	137	− 3
11	165	172	+ 7
12	210	220	+10
13	143	150	÷ 7
14	150	158	+ 8
15	154	165	+11
16	140	142	+ 2
17	158	170	+12
18	250	264	+14
19	160	155	− 5
20	140	140	0
21	145	152	+ 7
22	128	132	+ 4
23	139	145	+ 6
24	148	155	+ 7
25	180	190	+10
26	160	165	+ 5
27	190	185	− 5
28	154	163	+ 9
29	135	142	÷ 7
30	205	223	+18
31	160	154	− 6
32	150	150	0
33	142	150	+ 8
34	180	190	+10
35	135	140	+ 5
36	270	290	+20
37	155	160	+ 5
38	170	180	+10
39	310	325	+15
40	145	150	+ 5

ferent patients. The blood specimens were those sent to the laboratory for routine blood chemistry.

Table I gives the result of cholesterol determinations on forty specimens of blood.

As is shown in Table I, the new method gave slightly higher results than the Myers-Wardell method. This was to be expected, since the slightly lower results obtained with the Myers-Wardell method are due, as I pointed out before, to the loss caused by the transfer of material. Since in the new method no transfers of material are made at any time, no loss is possible.

To further facilitate the work of cholesterol determinations I have devised a special multiple condenser (Fig. 3) which allows six simultaneous extractions. The condenser is made of copper. Its diameter is 5 inches and its length 9 inches. It is held firmly by an iron tripod. Through the condenser pass six glass tubes. These tubes have a diminished diameter at each end. The lower end of each tube passes through a cork stopper which fits into the extraction tube. The upper ends are funnel-shaped through which may be added chloroform while in operation, if necessary. The glass tubes could easily be removed and replaced by new ones in case of breakage. The condenser is absolutely leak-proof.

The filter paper discs are especially prepared from very thick fat-free filter paper. They have a diameter of $\frac{3}{4}$ inch and a thickness of $\frac{1}{16}$ inch. The discs are perforated by a small hole so as to allow the escape of vapor into the condenser.

I wish to thank Samuel Gittlow and Milton J. Goodfriend, of the Lebanon Hospital, for their valuable suggestions and criticism, and for their checking up the accuracy of the method.

REFERENCES

[1] Grigaut: Compt. rend. Soc. de biol., 1910, lxvii, 791.
[2] Windaus: Ztschr. f. physiol. Chem., 1910, lxv, 110.
[3] Bloor: Jour. Biol. Chem., 1916., xxiv, 227.
[4] Autenrieth, and Funk: München med. Wchnschr , 1913, lxix, 1243.
[5] Myers, and Wardell; Jour. Biol. Chem., 1918, xxxvi, 147.
[6] Leiboff: Jour. Biol. Chem., 1924, lxi, 177.

URAN-GALLEIN, A NEW RAPID ELASTIC TISSUE STAIN PRODUCING SIMULTANEOUS STAINING OF NUCLEI AND PROTOPLASM*

BY FREDERICK PROESCHER, M.D., AND ALBERT PAUL KRUEGER, A.B.,
AGNEW, CALIFORNIA

FOR the selective staining of elastic tissue, we have two excellent methods, namely, that of Taenzer-Unna and that of Weigert. Both methods are absolutely dependable and stain the finest ramifications of the elastic elements, but possess in common the disadvantage of being too cumbersome for quick orientation.

In the course of staining experiments with gallein, we found that the latter in combination with uranium produced a mordant dye which is readily prepared and stains elastin in a few minutes with simultaneous staining of nuclei and protoplasm.

Gallein is dioxyfluorescein and is obtained by condensation of 1, 2, 3, trioxybenzol (pyrogallol) with phthalic acid anhydride (Bayer). It contains two OH groups in close relation to the chromophoric oxygen atoms, this molecular arrangement making possible the formation of a mordant dye with a metallic salt.

Gallein is not unknown to histologic technic but is used very little. In combination with aluminum sulphate or aluminum chloride, it is recommended as a good nuclear stain. It is marketed in the form of a violet paste or powder. It is practically insoluble in cold water, ether, benzol, or chloroform and dissolves in alcohol producing a dark violet color. With NaOH the solution turns blue and when treated with HCl develops a brown color. The dye is soluble in H_2SO_4 forming a red solution. Gallein treated with chromium or iron salts yields a beautiful violet-red mordant. The mordant prepared by treatment with aluminum sulphate has more of a purple tinge. If an excess of gallein is added to 2 per cent or 3 per cent alum solution or better a similar

solution of aluminum sulphate or chloride and the mixture is boiled for a few minutes a stain is produced which stains nuclei very distinctly.

PREPARATION OF THE STAINING SOLUTION

One and five-tenths gm. uranium acetate, 2.0 gm. sodium citrate, and 0.2 gm. sodium carbonate are dissolved in 50 c.c. distilled water. This solution will keep for three weeks or a month if placed in tightly stoppered bottles. It may be kept indefinitely if sterilized and placed in the dark. Light causes it to turn brown but does not in any way render it unfit for use. One-tenth gm. gallein (Merck) is added to 10 c.c. of the uranium solution, the mixture is boiled for two minutes and the solution cooled. The resultant purplish red solution will keep three or four weeks, if kept in the dark, and if desired may be filtered before use.

STAINING METHOD

The tissue should be fixed in formalin or better Muller-formol. For rapid work we prefer frozen sections from formalin or Muller-formol fixed tissue. However, embedding in paraffin or celloidin does not interfere with the staining.

The sections are immersed in the staining solution for five to ten minutes and washed with water (tap or distilled) for one or two minutes. If very fine differentiation is desired the sections should be washed in water for five to ten minutes. Prolonged washing does not alter the degree of elastic tissue staining but slowly decolorizes both protoplasms and nuclei.

For quick orientation the sections may be mounted in glycerin. They will keep for several days in that medium but slowly decolorize. For permanent mounting the sections, after washing in water, are dehydrated in absolute alcohol for three to four minutes, cleared in bergamot oil, and mounted in dammar balsam.

Counter staining is not necessary since nuclei and protoplasm are well differentiated. Nuclei are stained purplish red, protoplasm lavender, red cells deep brownish red and elastic fibers deep violet blue. Connective tissue appears light brownish red, muscle tissue deep brownish red to purplish red.

Immersion of the sections for one-half to one minute in a 0.25 per cent aqueous picric acid solution produces a yellowish brown to yellowish red differentiation of nuclei and protoplasm. After such treatment elastic fibers appear dark blue to blue black. If the sections are mounted in glycerin the picric acid treatment acts as a fixative preventing decolorization in the mounting medium.

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PRESERVATION OF ANTISHEEP AMBOCEPTOR WITH GLYCERIN*

By Virginia Langworthy, M.S., and Margaret Wemple, A.B., Albany, N. Y.

THE necessity for maintaining a supply of antisheep amboceptor sufficient for performing a large number of complement-fixation tests as well as for distribution to local laboratories throughout New York State led to a trial, in 1920, of the Clock and Beard[1] method of preserving amboceptor with 50 per cent of glycerin. It had been noted that in amboceptor sterilized according to the method previously followed, that is, heating for thirty minutes at 54° C. on three successive days, there is usually a decrease in titer after nine to twelve months' storage and it frequently becomes contaminated during use in spite of the attempt to observe strict aseptic precautions when withdrawing portions from the stock supply. The results of our four years' experience with the use of glycerin as a preservative for hemolytic serum have been so favorable that it seems important to add another endorsement to those which have already been published.[2, 3]

Comparative tests have been made with sterilized and glycerinated portions of hemolytic serums from ten different rabbits to determine the following points: (1) the effectiveness of glycerin in preventing the growth of common contaminants, (2) the rate of deterioration in the hemolytic titer of glycerinated amboceptor as compared with sterilized amboceptor, and (3) the effect of glycerin upon the complement-fixation reaction in syphilis.

EFFECTIVENESS OF GLYCERIN IN PREVENTING GROWTH OF BACTERIA AND MOULDS

Glycerinated portions of each of three amboceptors inoculated with B. subtilis, with a staphylococcus and a common mould showed no growth after four weeks at room temperature, while sterilized portions inoculated with the same cultures showed a heavy growth after five to six days at room temperature. The fact that glycerin prevents the growth of common contaminants, which was thus experimentally demonstrated, has since been fully confirmed by the use of glycerinated amboceptor for routine work, during three years.

COMPARISON OF HEMOLYTIC TITER OF THE STERILIZED AND GLYCERINATED
AMBOCEPTORS AT THE END OF THE STORAGE PERIODS

In testing the hemolytic titer at intervals during storage, the titrations of both the sterilized and the glycerinated portions of each amboceptor were made on the same day, and with the same complement and the same suspension of sheep cells.

After four years' storage at 3 to 6° C., nine of the ten glycerinated serums showed no change in titer. The other serum preserved with glycerin showed a slight decrease in titer at the end of eighteen months. Of the ten sterilized serums, only four showed no change in titer at the end of four years. Five serums showed a slight decrease in titer, one at the end of nine months, three at the end of three years, and one at the end of four years; one showed a marked decrease in titer at the end of four years. It might be added in this connection that a large supply of antisheep amboceptor produced in a mule, in

1921, was preserved with glycerin at that time and has shown practically no change in titer up to the present.

Not only did the glycerinated portions show less deterioration in titer, but they contained less precipitate than the sterilized portions, after long periods of storage. No constant relation was observed, however, between the formation of precipitate and loss of hemolytic activity, as two of the sterilized serums which showed no decrease in titer contained a large amount of precipitate.

EFFECT OF GLYCERIN UPON THE COMPLEMENT-FIXATION REACTION

In a large series of complement-fixation tests, cells sensitized with glycerinated amboceptor of 1:1000 titer† gave results agreeing very closely with those of routine tests in which cells were used which had been sensitized with amboceptor containing no glycerin. The few differences which did occur were slight and within the limits of technical inaccuracies.

Since a high concentration of glycerin would result in the test if 50 per cent of glycerin were added to an amboceptor of low titer, experiments were made to determine the maximum amount of glycerin which could be present without influencing the complement-fixation reaction. When the concentration of glycerin in the diluted amboceptor was as high as 1:10, slightly more complement was required for hemolysis, and when the concentration was from 1:10 to 1:50, some lysis of the sensitized cells was evident if they stood for two to three hours at room temperature. The use of amboceptor containing these concentrations of glycerin did not affect the complement-fixation reactions, however, when the appropriate amount of complement was used and the cells were added to tests fifteen minutes after sensitization. When the concentration of glycerin was only 1:100 or less, there was no appreciable effect upon the cells or upon the results of complement-fixation tests. Since an amboceptor of titer lower than 1:100 would not ordinarily be used, the effect of the amount of glycerin introduced through amboceptor preserved with 50 per cent of glycerin appears to be negligible.

CONCLUSION

The results of these experiments have indicated that the addition of 50 per cent of glycerin is a practical and efficient method of preserving amboceptor. It is effective in preventing the growth in serum of common contaminants; the rate of deterioration in the hemolytic titer of glycerinated amboceptor is, in general, less than that of sterilized amboceptor, the titer of the former remaining unchanged for a period of at least four years; and 50 per cent of glycerin added to a hemolytic serum of reasonably high titer has no influence upon the complement-fixation reaction in syphilis.

REFERENCES

[1]Clock, R. O., and Beard, S. D.: Preservation of Antisheep Hemolytic Amboceptor in Glycerol, Jour. Infect. Dis., 1917, xxi, 404.
[2]Kolmer, J. A., and Rule, A.: A Study of Methods for the Preparation and Preservation of Hemolysins, Am. Jour. Syph., 1920, iv, 484.
[3]Kilduffe, Robert A.: A Note upon the Stability of Preserved (Glycerinated) Antisheep Amboceptor, JOUR. LAB. AND CLIN. MED., 1924, ix, 651.

†The titer is expressed in terms of the dilution which contains two units in 0.1 c.c. Since 0.1 c.c. of diluted amboceptor is used in a test having a total volume of 0.5 c.c., the final concentration of glycerin in the test is only one-fifth as great, e.g., in this instance, 1:5000.

A NEW BACTERIOLOGIC STAIN*

BY L. E. HARRISON, PRESCOTT, ARIZONA

THE stain to be described was developed following the observation that formalin added to an aqueous solution of basic fuchsin containing phenol, increased its fastness for bacteria, and after standing for two weeks, changed from deep red to a dark purple. After some experimentation, the writer worked out the following formula:

One gram of basic fuchsin is added to 100 c.c of distilled water and gently heated to 100° C. The solution is then filtered. To 75 c.c. of filtrate, 10 c.c. of 37 per cent formaldehyde, 10 c.c. of saturated aqueous solution of phenol, and 5 c.c. of glycerol are added. The stain is now placed in a glass stoppered bottle and allowed to stand for two weeks. At the end of that time it appears that polymerization of the fuchsin in the presence of formaldehyde has taken place with the production of a dark purple stain. The stain seems to become darker on standing, and improves in staining quality. Deterioration does not take place.

For staining tubercle bacilli, a few drops of the stain are added to the smear and heated over a flame until steaming. The slide is then set aside for about four minutes. At the end of this time the smear is decolorized with an alcoholic sulphuric acid solution (15 parts of concentrated sulphuric acid to 85 parts of 95 per cent of ethyl alcohol). The smear is now washed with tap water and counterstained for two minutes with a saturated alcoholic solution of picric acid to which has been added 5 c.c. of Lugol's solution for each 95 c.c.

A NEW STAIN

The tubercle bacilli stain with remarkable clearness as brownish black rodlike forms against a light brownish yellow background. Beaded forms are particularly prominent.

The organisms of Vincent's angina are readily stained with this method. The smear is stained in the same manner as for staining the tubercle bacilli. It is then decolorized with 95 per cent alcohol, and counterstained with the picric acid iodine solution. The spirochetes stand out as dark spirals against a yellow background.

Other organisms may be stained with this method. Gram-positive bacteria are most suitable. The diphtheroid group seem to be especially well stained. Spore forms are also well stained.

A NOTE ON THE USE OF THE SWIMMING CUP FOR WASHING TISSUES*

By Elizabeth Morford, B.A., Houston, Texas.

FOR efficiency in washing tissue free of fixing solution, float it in a swimming cup. This is a porcelain cup or thimble with perforations in the sides and fitted with a cork stopper which floats the cup in the washing fluid.

If the cup is set to wash in a half liter beaker under a slowly running faucet, the current produced in the water is strong enough to keep the cup jostling and bobbing and the holes in the cup are large enough to allow the water to run through the cup freely.

There is no danger of the piece of tissue being lost and there is the assurance that it is constantly being bathed in fresh water. The cup in the large size nicely accommodates several blocks of tissue that have been sampled from the gross block.

The swimming cup was planned originally for washing specimens of ore. It will be found illustrated in the Arthur Thomas catalogue.

Communication

In the January issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, there appeared an article by C. E. Reynar, entitled, "The Red Corpuscle Suspension for the Kolmer-Wassermann Reaction."

We wish to draw attention to the fact that we published an article entitled "The Use of Formalinized Sheep Cells in Complement-Fixation Tests" which appeared in the Zeitschrift für Immunitätsforschung und Experimentelle Therapie for June 1912.

In this article we covered the subject of Dr. Reynar's work completely, arriving at similar conclusions.

Since that time our article has been quoted a number of times and the method of preserving sheep cells for complement-fixation tests originated by us has been in use in our own and in other laboratories.

We are greatly surprised that Dr. Reynar overlooked our article and we wish to take this opportunity of drawing the attention of your readers to our work which antedated Reynar's by so many years.

Yours very truly,

(Signed) Bernstein and Koliski.

New York, May 19, 1925.

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*From the Southern Pacific Hospital, Houston, Texas.
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EDITORIALS

Cervical Sympathectomy in Angina Pectoris

MOST people first consult their physician for the relief of pain or discomfort. Their primary interest is in obtaining such relief and he who can accomplish this most readily and satisfactorily is considered the best physician. Nondescript pain will usually respond to routine therapeutic measures and often the physician relieves the pain before he has had an opportunity to study it, to learn of its origin or mode of action. Volumes have been written on pain alone and yet our knowledge of this symptom is still quite superficial. The experimental difficulties are obvious. A person suffering pain of any degree will not consent long to a scientific study while feeling that proper therapeutic measures would give prompt relief. The experimental animal can only describe his pain in a general way. Besides, the principles of humaneness deter us from inflicting any considerable unnecessary pain.

The victim of angina obviously desires, foremost, relief from the excruciating pain. Relief at best is usually temporary. It. is, therefore, not surprising

that the work of Jonnesco, first reported in 1916, and revived by Brown and Coffey, in 1923, has received considerable interested study and the enthusiastic support of many. Cervical sympathectomy usually relieves the pain of angina, often permanently.

Various writers, particularly Sir James MacKenzie, whose authority it would be presumptuous to ignore, have questioned the advisability of removing a danger signal whose absence may result direly. Pain notifies when the heart is being overworked and forces the victim to stop all exertion. It is in part at least a protective measure.

Morphine is one of the most valuable drugs for relief of pain. It is often clearly indicated and without doubt has been life saving; yet we would display the poorest sort of judgment were we to use morphine for all pains. Likewise in angina, cervical sympathectomy relieves pain. It appears probable that in certain types of angina or in angina attributable to certain causes, the operation will be found to have a legitimate therapeutic place. The work is too recent for a clear-cut statement of operative indications, but since it is apparently coming into rather general use, since it is readily agreed to by the patient, and since it opens a new field for surgical hyperenthusiasm, it is well to study the work done so far, in an attempt to find some broad general lines to follow.

The first observation after operation and naturally the most striking is the immediate and usually complete relief from pain. The second observation gained from a study of the literature is that while the results are admirable, some patients die relatively soon after recovery from operation. They die without pain to be sure, but experiencing the familiar sensation of thoracic tightness and anxiety with fear of impending dissolution.

Generalizing, we may say that where there is evidence of extensive and grave myocardial damage the operation removes a valuable warning mechanism and predisposes to a relatively early death from unexpected overexertion. Thus, one patient about two weeks after convalescence from a successful operation, had talked with the physician making his rounds and was in excellent state. The doctor having left the room, the patient recalled a question which he wished to ask, jumped up and hurried into the hall. He suddenly experienced an intense uneasiness and anxiety, and with no pain whatsoever, promptly died. In cases with long-standing advanced myocarditis with or without auricular fibrillation or valvular disease, the advisability of operation is open to grave question. If it is performed, the patient's activities must be controlled with a degree of care and over a period of time, difficult or indeed impossible to accomplish. Where angina is the result of recurrent coronary thrombosis with infarction, grave myocardial damage must of necessity be present.

However, true angina pectoris not infrequently occurs in individuals in whom remarkably little physical or roentgenologic evidence of cardiac damage is observed. As time goes on we will probably find that the operation will be applied particularly in this group. MacKenzie, however, has stressed that such individuals have often been relieved for years by more conservative methods. The rationality of cervical sympathectomy will depend ultimately upon further

elucidation concerning the cause of angina. This will naturally lead to the classification of the disease into several different groups.

MacKenzie states that angina is still a matter for vague speculation and that the employment of such drastic treatment as an operation for a condition so little known does not redound to the credit of medicine. True, the operation is in a sense empiric but the results are definite and the same criticism might equally apply to many other successful surgical procedures. He points out that pain and other symptoms associated with angina pectoris are not infrequent in people whose hearts are healthy, but who suffer from ill health which disturbs the nervous system. The majority of these are women under fifty years of age. During the war numbers of young soldiers showed all the signs of angina pectoris but they suffered from some infection often too obscure for identification but which in some instances was ultimately found to be trench fever. He classifies these as "secondary angina." Such cases, followed by MacKenzie for many years have in no instance died from an affection of the heart. On the other hand, in the majority of cases of primary angina, morbid changes are found at necropsy in the coronary arteries and in the muscles of the left ventricle. These consist of narrowing of the lumen in some part of the coronary arteries such that the supply of blood to the muscle is sufficiently diminished to impair functional efficiency and to cause degeneration and destructive changes in the heart muscle. The healthy coronary artery is very distensible and readily accommodates great increase in the blood supply when the heart is called upon to increase its work.

Wiggers calls attention to the milking action of the heart muscle serving to increase the coronary blood supply. The heart does not beat during perfusion with an oxygen-free solution. If adrenalin be added to the solution, the flow through the coronaries is *decreased* from constriction to the peripheral vessels. On the other hand, a heart perfused with oxygenated Ringer's solution continues to beat and adrenalin this time *increases* the blood flow, since it simultaneously increases the rate and amplitude of ventricular contraction, which is sufficient to overpower the constrictor effect upon the coronary vessels themselves. Such milking action ceases to exist when the arteries become hardened by sclerosis or calcification, or obliterated by endarteritis.

While the pathologic picture in angina is usually that of coronary sclerosis, many persons come to autopsy with coronary sclerosis but who have never, as far as can be ascertained, experienced the symptoms of angina. Sclerosis without angina may exist, but true primary angina probably does not occur without some coronary involvement. Sclerosis alone, according to MacKenzie, is probably insufficient to account for angina. Nearly always some myocardial impairment accompanies the vascular change.

What causes the pain of angina? What occurs within the heart synchronous with the pain? In general, three explanations have been seriously considered. The condition may be associated with a deficient blood supply. Intermittent claudication in the peripheral muscles, likewise associated with arteriosclerosis, results from local anemia and is accompanied by intense pain. The two diseases may be quite similar.

A second theory and one which we may consider as corollary to the first, is that during the anginal attack, a spasm occurs in vessels already narrowed by sclerosis. Vasoconstriction is not a necessary part of the first hypothesis in that with coronary sclerosis the blood supply may be diminished without concomitant spasm. Vasomotor fibers have been shown to exist in the coronary vessels but it has not been demonstrated that constriction of these vessels is the cause of angina.

Sir Clifford Allbutt associated the pain with stretching of the aorta which produces pressure on the sensory end organs in the adventitia of this vessel.

Considering the more generally accepted theories, pain is admittedly associated with muscular spasm. Myocardial spasm may result from inadequate blood supply. But what produces the pain? Is it attributable, as has been suggested, to chemical stimulation of accumulated waste products in the muscle cells, or to pressure of the spastic muscle on sensory nerves? Some hold that there are no sensory nerves in the heart. Pain is then explained as a visceromotor reflex, the afferent stimuli of which so alter the irritability of those spinal cord segments from which its nerves arise that the pain is referred to the skeletal sensory nerves having their terminals in the same segments. This would explain the distribution of the pain through the chest and arm. Along with the viscerosensory reflex a visceromotor reflex is set up with resulting constriction of the intercostal muscles. According to the hypothesis, this visceromotor reflex accounts for the sense of thoracic constriction.

While coronary embolism or thrombosis usually is associated with a more lasting pain and is more often fatal during the acute attack, recurrent small thromboses have been responsible for typical recurrent angina, lasting over a relatively long period.

Coming again to cervical sympathectomy, MacKenzie has taken rather vigorous opposition to the procedure. All that the surgeon could hope to accomplish, he says, would be to cut the nerves which convey those impulses giving rise to pain, from the damaged heart to the center of consciousness. He believes that this is not for the best interest of the patient. Pain is a signal which is given out by a muscle when exhausted and particularly when forced to work with a defective blood supply. The failure to recognize pain may lead the individual to persist in efforts beyond the heart's capacity. He affirms that while the pain accompanies a condition which is dangerous, the pain in and of itself is not dangerous.

Shortly after the publication of MacKenzie's criticism, J. N. Langley presented a communication supporting MacKenzie's contention. He stated that the theory on which most of the operations have been made is that a considerable number of sensory fibers of the heart lead to the central nervous system by way of the cervical sympathetic and vertebral nerves. He describes several anatomic and physiologic studies which would show that severing of the sympathetic trunk cuts no appreciable number of afferent fibers.

Granting that few if any sensory nerves are cut during removal of the cervical sympathetic ganglia, why is it that as obviously occurs, the pain is relieved?

Ransom agrees with Langley in his observation that no sensory nerves are found in the upper portion of the sympathetic tract. He has traced sensory nerves from the heart as high as the middle cervical ganglion but from here and from the lower cervical ganglion they pass over with the cardiac branches of the vagus, to the vagus nerve and ascend with it. It appears quite clear that particularly where the superior cervical ganglion alone is removed, as in the operation recommended by Coffey and Brown, no noteworthy sensory nerves are divided. Unlike Langley, however, Ransom and Holmes do not look upon this as a reason why the operation should not be performed. Recognizing that some change must have taken place whereby the patient was relieved of his most distressing subjective symptom, they hypothecate that in the operation, efferent nerves, probably vasoconstrictor, have been divided. Wiggers has shown that stimulation of the vagus nerve causes constriction of the coronary arteries. Holmes and Ransom believe that the vasoconstrictor fibers found by Wiggers in the vagus have their origin in the superior cervical sympathetic ganglion. They quote certain embryologic data tending to support this view. While it may be that, as suggested, the beneficial results from superior cervical sympathectomy come from dividing efferent rather than afferent nerves, we have ample evidence that considerable disturbance in various sensory nerves has been effected by the operation. The pain above the left scapula described by various writers, the jaw pain at the beginning of mastication, trigeminal neuralgic symptoms, are clearly sensory symptoms. In the more extensive operation done by Reid, a few cases have developed more marked sensory changes in the left half of the body from the head to the costal margin, with absence of epicritic sensibility, lowered threshold for pressure pain and for temperature, diminished protopathic sensibility, disturbed stereognosis, etc.

Reid's suggestion, that without clinically recognizable evidence of cardiac or aortic disease angina may be analagous to trigeminal neuralgia, is worthy of consideration. In such cases removal of the cervical ganglion would correspond to removal of the semilunar ganglion in tic douloureux. Brunning has reported pathologic changes in the sympathetic ganglia of a case of angina who was relieved by operation. These changes consisted of tissue increase, thick walled vessels, perivascular and intercellular lymphocytic infiltration, and enlarged ganglion cells apparently involved in degenerative changes.

Summarizing the evidence reviewed, we may say that there is justification for operative procedure in some cases of angina, particularly those with little physical or roentgenologic evidence of degenerative disease of the heart, aorta, or peripheral vessels. Reid and Freidlander report a case with sudden death in whom the physical findings showed little evidence of cardiac pathology, but the patient had considerable pulmonary emphysema and from the location of the cardiac impulse it seems probable that fluoroscopic examination would have shown marked cardiac hypertrophy. In all probability there was considerable myocarditis in this case. In properly selected cases the results have occasionally been spectacular. Bacon's patient seventy-eight years old, was able to walk three miles at a time without resting and without discomfort. Diez describes a man forty-two years old who had suffered from angina for eleven years, with

attacks increasing so that at the time of operation he was having an average of three a day. Since the operation he has had no pain, nor dyspnea even after an eleven mile walk.

The case must be most carefully selected from the viewpoint of cardiac pathology. The internist must decide as to the advisability of surgical intervention and must follow the case most carefully thereafter. In those so far reported not sufficient attention appears to have been given to electrocardiographic studies. Thus, an individual under the personal observation of the reviewer, with characteristic true angina but with no evidence on physical or fluoroscopic examination of noteworthy myocardial, aortic, or vascular damage, showed an inversion of the T-wave in lead two, indicating distinct myocardial change. We feel that in the preoperative and postoperative study of these cases, the electrocardiograph will find a wide field of usefulness and that its application will ultimately be of help in selecting the suitable case.

While little claim has been made for the operation beyond palliative effect and indeed little further can be claimed until more is learned of the innervation of the heart, the work reviewed suggests that we may find a truly curative tendency in a small proportion of the cases. Many cases come to autopsy with sclerosis of the coronary vessels who have never experienced angina. If in addition to sclerosis, an angiospasm is requisite to the development of angina and if operation, by severing the efferent fibers prevents this spasm, we may expect most excellent results in those cases where the cardiac lesion is limited to coronary sclerosis. Such strict limitation, however, is probably not common

REFERENCES

Brown, P. K., and Coffey, W. B.: Arch. Int. Med., 1924, xxxiv, 417
Diez, J.: Jour Am. Med. Assn., 1924, lxxxiii, 879
Florcken, H.: Jour. Am. Med. Assn., 1924, lxxxiii, 956.
Holmes, W. H., and Ransom, S. W.: Jour. Lab and Clin. Med., 1924. x, 183,
Langley, J. N.: Lancet, London, 1924, cvii, 955.
Lilenthal, H.: Arch. Surg., 1925, x, 531.
MacKenzie, Sir James: Lancet, London, 1924, cvii, 695.
Neuhof: The Heart, 1923, p. 407.
Ranschoff, J. L.: Ann. Surg., 1925, lxxxi, 585
Reid, M. R., and Andrus, W. D.: Ann. Surg., 1925, x, 591.
Reid, M. R., and Freidlander, A.: Jour. Am Med Assn , 1924, lxxxiii, 113.
Reid, M. R., and Eckstein, J.: Jour. Am. Med. Assn., 1924, lxxxiii, 114.
Wiggers: Circulation in Health and Disease, 1923, p 431.

—W. T V

The American Society of Clinical Pathologists

State Pathological Society of Texas

The ninth annual meeting of the State Pathological Society of Texas was held in Austin, May 4, 1925.

The following papers were presented:

President's Address.   By Dr. B. F. Stout, San Antonio.

Is the Pathologist Properly Appreciated?   By Dr. M. E. Bledsoe, Port Arthur.

Syphilitic Aortitis with Presentation of Specimens.   By Dr. Willis W. Waite, El Paso.

Cardiac Infarcts.   By Dr. G. Werley, El Paso.

Basal Metabolism Determination.   By Dr. J. H. Black, Dallas.

Tumors of the Nervous Tissue of the Adrenal Body.   By Dr. Henry Hartman, Galveston.

Primary Carcinoma of the Liver, with Report of Four Cases.   By Dr. W. H. Hill, San Antonio.

Pernicious Anemia.   By Dr. J. G. Burns, Cuero.

CLINICAL AND EXPERIMENTAL

PERNICIOUS ANEMIA*

By John W. Shuman, M.D., F.A.C.P., Los Angeles, California

HERETOFORE all anemias, including those presenting a pernicious anemic picture, were labeled secondary when a definite cause was found. In the anemia in which no cause was found, and presenting the pernicious anemic picture, the diagnosis of a primary anemia always held. Pernicious anemia as a diagnostic entity should be relegated.

Considering pernicious anemia from all angles it is difficult to accept any view other than that it is the result of a *defense reaction* of the body, as a whole, against toxemia; a long continued fight finally resulting in a state of chronic invalidism, leading to death. Its cardinal symptoms are grave anemia, hemosiderosis, general weakness, moderate loss of weight, tachycardia, fever, gastrointestinal, visceral, and cord changes. Its course is marked by exacerbations and remissions.

Reviewing recent literature, the facts stand out clearly that:

1. The cause is not understood.
2. Its recognition is not early enough.
3. Its symptomatology is not rationally explained.
4. There is no logical linking of its cause and course.
5. Its treatment is still symptomatic and empirical.

ETIOLOGY AND PATHOGENESIS

William Hunter[1] considered "long standing oral and gastric sepsis" to be the cause of pernicious anemia. Sepsis is a cause but there are other contributing factors; for example, environment, pregnancy, cancer, endocrine dis-

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anemia, can be explained by a long continued sympathetic stimulation causing inhibition of the gastric secretion, not only hydrochloric, for there is also an inhibition of intestinal secretions and motility causing atonic constipation and periodic diarrhea.

Gastrointestinal tract changes are those encountered in toxemia, viz., gingivitis, stomatitis, glossitis, gastritis, enterocolitis, and proctitis. These are most marked during exacerbations. They may precede and usually improve during a remission.

The visceral changes, enlarged spleen, and liver, occur in all cases. Albuminuria is frequent.

The hypertrophy of the spleen is due to overactivity making good in the rôle it was created for, viz., ridding the blood stream of debris; in this instance a malicious thing, for it destroys the immature type of red blood cell,—the only blood cell the patient has: hence splenectomy has been popularized because it extends the patient's life for a few more years in the majority of instances.

COURSE

Each exacerbation leaves the patient in a more precarious state. Finally, exhaustion of the hematopoietic system, as is witnessed in that individual who completes the course, takes place. There is no doubt but that many die from the acute toxemia and are never permitted to reach the stage of bone marrow exhaustion; while others recover without reaching this stage; leaving a third class, necessarily the minority, to develop the grave anemia.

In pernicious anemia, the basal metabolic rate is usually increased, showing that the oxygen carrying capacity of the hemoglobin is not lessened. The lung ventilation remains the same. The total amount of hemoglobin is absolutely lessened due to a diminished cell count and diminished blood volume. This is partially compensated for by tachycardia and malaise. Exertion causes pallor, shortness of breath, vertigo, and excessive tachycardia.

For discussion, three case records are submitted, the one in the abstract, for he is dead, the other two are still with us.

CASE 1.—Demonstrates the following symptoms: (1) early nervous; (2) gastrointestinal; (3) blood system changes; (4) visceral evidence of hemolysis; (5) progress to fatal termination.

No. 4325, W. S., age thirty-four, admitted to the service of W. C. Allison, M.D., Feb. 15, 1924, complained chiefly of "pain in the upper abdomen." His father died from "pernicious anemia (?)." The patient's sister stated that he had a "number of attacks of mental disturbance (amnesia), following his discharge from the service, June, 1919''; in 1920, he had a definite attack of persistent diarrhea; a gradual failing in health for the last one and one-half years with his color becoming more sallow and yellowish, accompanied by upper abdominal pain. One year prior to his admission here, he had an attack of abdominal colic, jaundice, and clay colored stools.

Physical examination.—Height, 70 inches; weight, 150 pounds (usually 165); color, sallow; mentally, apathetic; heart, soft systolic murmur at base; tender gall bladder area; spleen, palpable; internal hemorrhoids; cord changes were evidenced by rectal and urinary bladder incontinence, painful cramps and weakness of lower limbs, and absent knee-jerks.

Laboratory findings.—Urine, specific gravity, 1.026; hyaline, fine, and coarse granular casts; numerous pus cells and an occasional red blood cell. Blood, red blood cells, 1,912,000; hemoglobin, 40 per cent; color index, one-plus; blood platelets, 104,000; following transfusion, red blood cells, 2,300,000; white blood cells, 5,000; hemoglobin, 58 per cent; color index, one-plus; further transfusions, and on May 1, red blood cells, 2,072,000; white blood cells, 5,200; hemoglobin, 52 per cent; color index, 1.37; marked delle, poikilocytosis
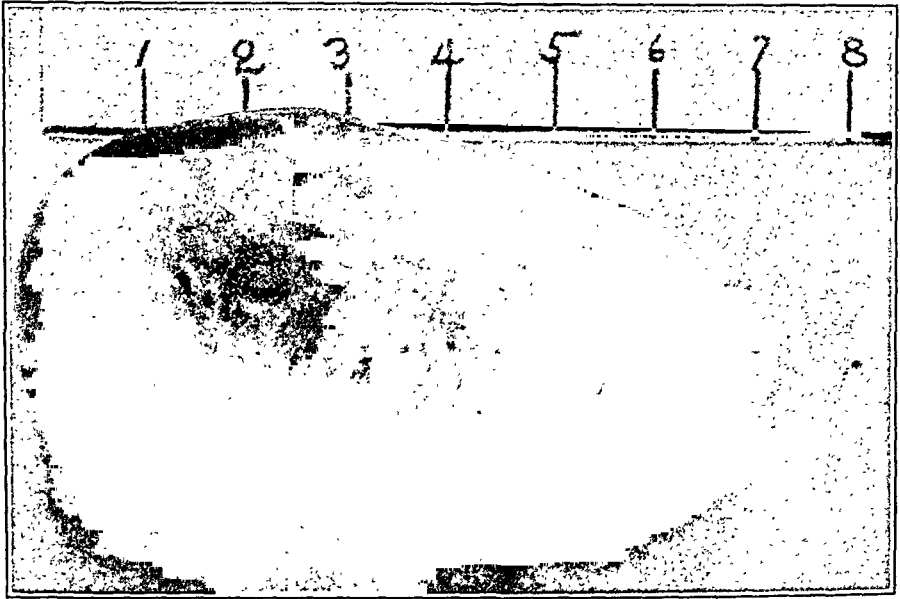
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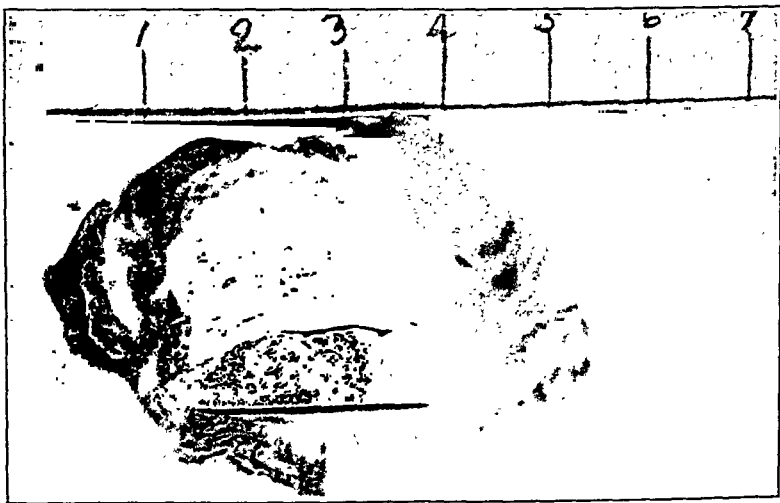
Fig. 2.

and anisocytosis; Wassermann, negative. Gastric analysis, gastrointestinal x-ray series, and spinal puncture were not done on account of patient's lack of cooperation.

A working diagnosis of ''pernicious anemia, due to chronic toxemia,'' was made. The gall bladder, at operation, by James A. Mattison, M.D., was found to be, ''definitely and chronically infected''; it was drained. His progress notes from then on to September 6, when he died, show, ''progress from bad to worse.''

Autopsy by W. C. Allison, M.D., revealed: "(1) old adhesions between gall bladder and duodenum; (2) a greatly thickened pylorus; (3) chronic appendicitis; (4) hypertrophic cirrhosis of liver; (5) splenomegalia [See Fig. 1]; (6) fibrotic pancreatitis; (7) diverticula of urinary bladder." [See Fig. 2.]

It is regretted that permission for a complete autopsy was not obtained.

CASE 2.—Demonstrates: (1) cord; (2) gastrointestinal; (3) blood; and (4) visceral changes.

No. 6081, F. A. F., age forty-five years, admitted to service of G. S. Craig, M.D., Sept. 20, 1924, complained of "General weakness, especially in legs, and gastric disturbance."
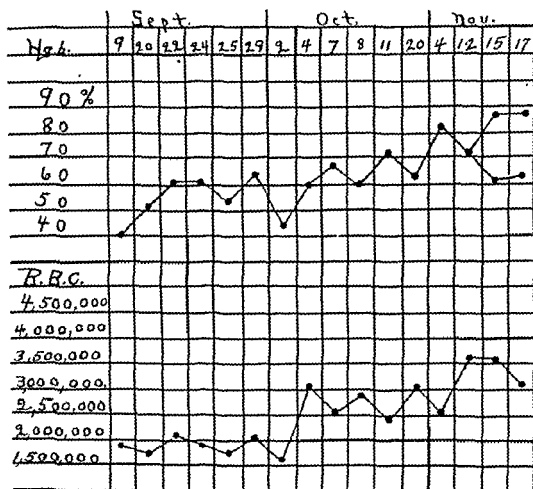
Fig. 3

CASE 2. September 9. No nucleated red cells or myelocytes; marked anisocytosis and poikilocytosis.
September 22. Autohemic therapy.
September 26. Autohemic therapy.
October 4. Autohemic therapy.
October 11. Slight anisocytosis and poikilocytosis. Slight central depression of R. B. C.
November 12. No pathologic white cells found, polymorphonuclears predominating type of cell, no nucleated reds. Cells staining more uniformly, and showing very little poikilocytosis and anisocytosis.
November 13. Autohemic therapy
November 15. No pathologic white cells, slight anisocytosis and poikilocytosis. Red cells uniformly stained.

His father died at age of sixty-three, from "uremia." He had "typhoid-malaria and yellow fever in 1899"; in January, 1924, a hemorrhoidectomy was performed.

Physical examination.—Undernourished, feeble man, with marked pallor; thyroid, one-plus; exophthalmus, slight; slight lagging of upper lids; slight wrinkling of forehead; the backs of the neck and hands *markedly* pigmented, this has been present since the "yellow fever"; hair, scant; six lower remaining teeth in fair condition (pyorrhea cause for loss); nose, right septal spur; tonsils, chronically infected; right inguinal hernia; still has protruding and bleeding hemorrhoids, a spastic sphincter, and hypersensitive rectum; a slight varicocele on the left; marked weakness and numbness in both legs and feet; a marked Rhomberg; gait, ataxic; absent knee jerks; tender lumbar muscles.

Laboratory findings.—Red blood cells, 1,710,000; white blood cells, 4,600; hemoglobin, 52 per cent; color index, one-plus; marked aniso- and poikilocytosis; Wassermann, negative; refuses spinal puncture; stomach, hydrochloric deficit of 37, combined acids 9; urine, a few pus cells; x-ray findings by R. C. Shawhan, M.D., reports, "evidence of duodenal ulcer; irregular hypomotility; and chronic colitis."

A working diagnosis of "pernicious anemia due to chronic toxemia," was made. His focal infection has been removed. Rest, autohemic and hydrochloric therapy seem to have improved him clinically for a remission is establishing itself (Fig. 3). Blood count Nov. 15, 1924, red blood cells, 3,780,000; white blood cells, 3,600; hemoglobin, 80 per cent; color index, one-plus; blood platelets, 310,000; poikilocytosis and anisocytosis, slight; cells stain uniformly.

CASE NO. 3.—No. 5814, L. W., age thirty-seven, admitted to the service of W. C. Allison, M.D., June 26, 1924, complained of "weakness."

Father died from "kindey disease"; mother from "cancer of womb"; patient operated upon in 1919, in France, for rectal fistula and hospitalized for five months following, for "weakness." There seems to have been a remission, for it is not until December 1923, that the next hospital record appears; when it was discovered that he had but 500,000 red blood cells.

Physical examination.—Height, 68 inches; weight, 133 pounds; a marked lemon-tint pallor of the skin; slight but general lymph gland enlargement; hearing, defective; tonsils, diseased; nose, right deflected septum; gums, spongy and bleed easily; two apical abscesses of teeth; heart, systolic murmur at base; tender gall bladder area; a stricture of urethra; fissure of rectum.

Laboratory findings.—Urine, faint trace of bile, indican, and a moderate number of pus cells present. Blood, red blood cells, 560,000; white blood cells, 3,500; hemoglobin, 30 per cent; color index, 2.6; blood platelets, 104,000; coagulation time, seven minutes forty-five seconds; Wassermann, negative; bilirubin index of blood serum, 40 (normal 10 to 15); fragility test, normal; stomach, total acidity 10, with hydrochloric deficit of 18.

X-ray series of gastrointestinal tract by R. C. Shawhan, M.D.: "gastroptosis, irregular motility, caecal ptosis, and colonic angulation."

SPINAL FLUID ANALYSIS

Progress notes.—September 16, tonsils removed by Arthur E. Gill, M.D., cultured and hemolytic streptococci grew. Frequent gastrointestinal disturbances have occurred (vomiting, diarrhea, and abdominal pain), although dilute hydrochloric acid 10 to 20 drops t. i. d. seems to control. Frequent transfusions of 450 c.c. of blood from various donors have been given with apparent beneficial results. Blood findings Nov. 16, red blood cells, 2,300,000; hemoglobin, 81 per cent; color index, 1.76; aniso-and poikilocytosis, slight; repeated counts show the white blood cells never over 4,600, and never below 3,500 with the decrease in polymorphonuclear cells constant.

In Cases 2 and 3 all focal infection has been eradicated; rest has been maintained; transfusion and autohematization have been performed as often as we thought wise and here we are with a remission evidently taking place.

TREATMENT

Although a chapter in itself, it is briefed here:

1. Rest: this cannot be overstressed for obvious reasons; it lessens katabolism and damage to the vital organs, especially the heart.

2. The detection and removal of all focal infection:

(a) chronic upper respiratory (sinus, etc.) infection;

(b) chronic oral (gum, tooth, tonsil, tongue, etc.) infection;

(c) gastrointestinal and accessory visceral (stomach, gall bladder, pancreas, small and large gut, appendix, rectum, etc.) infection;

(d) genitourinary and reproductive tract, infection.

3. Hemietherapy:

(a) Transfusion: often gives a supply of a number of red blood cells equal to two-thirds of the patient's total count. These cells have been shown to survive a sufficient length of time to warrant this form of treatment; it may lessen hemolysis. Much care should be exercised in the selection of a donor in this class of patients, picking one that is free from not only acute but chronic infection, syphilis not among the greatest.

(b) Autohemic therapy for the production of antihemolysis with the advantage that it is easy to procure and in some instances the results have not hindered a remission.

5. Arsenic, diet, fresh air, and sunshine. Arsenic probably lessens hemolysis by depressing the endocrine system.

Diet should be nutritious and easily digestible; and dilute hydrochloric acid gtts. q.s. t.i.d. p.c. to supply the deficiency.

6. Splenectomy should be done:

(a) after all focal infection has been eradicated;

(b) after transfusing has lessened hemolysis; and

(c) during a remission when the blood is in a fair condition.

Splenectomy eradicates an important red cell destroying agent, thus preserving the immature cells, the best the patient can produce.

Regarding autopsy in "pernicious anemia," it is an old story to hear the remark, "Autopsy findings clearly demonstrated pernicious anemia." I offer the suggestion that that is what the pathologist set out to see, the effect rather than the cause. Thoroughgoing autopsies like physical examinations are few and far between. Few autopsies embrace a search for focal infection or a study of the endocrine glands. The fault lies at the clinician's door, for he lacks interest, not only in securing autopsy material but failure in witnessing and assisting the pathologist in his search.

An early recognition is insisted upon; grave or severe anemia, instead of "pernicious," as a diagnostic entity should be used, for these patients have learned that our medical forefathers and most of our brothers diagnose and treat pernicious anemia as "hopeless."

SUMMARY

1. Pernicious anemia has a cause, a long standing toxemia.

2. This toxemia is usually due to bacteria, causing a high grade hemolysis, which exhausts the blood-forming system.

3. It is the duty of the physician to accept this dictum to find and remove, if possible, cause or causes, the first step in treatment.

4. Following or failure of No. 3, the treatment is symptomatic.

REFERENCES

[1]Hunter, William: Severest Anemias, New York, 1909, Macmillan Co.
[2]Stitt: Bacteriology, Blood Work, Parasitology, Philadelphia, 1923, P. Blakiston's Son and Co., p. 677.

THE ACTION OF NITRITES UPON PULMONARY CIRCULATION*

I. NITROGLYCERIN

By George R. Love, M.D., and Hugh McGuigan, M.D., Chicago, Ill.

THE present communication deals with the effect of nitroglycerin upon pulmonary arterial pressure and excised vessels, and attempts to explain the apparent contradiction in the effects on the pulmonary and other vessels. Macht[1, 2] in his studies of the action of various drugs upon excised pulmonary arteries found that the nitrite group caused a contraction of these vessels. This action is unusual in view of the commonly accepted statement that the nitrites are smooth muscle depressants.

Bradford and Dean,[3] Wood,[4, 5] and Petitjean[6] observed that the nitrites increased pulmonary arterial pressure. Bradford and Dean attributed the mechanism to "back pressure" from defective cardiac action. Wood and Petitjean believed that nitrites produced active constriction of the pulmonary vessels, and the latter substantiated his belief by observing a blanching of the lungs, particularly evident following amyl nitrite.

The method used for excised pulmonary arteries has been described by Macht.[1, 2] Dogs were used in the present investigation, including the experiments with excised vessels. For anesthesia several methods were used: ether first, then decerebration; ether first, then urethane; urethane alone. No difference was observed in the anesthetics except through their influence upon central vagal and vasomotor tone. Pulmonary pressure was recorded by the methods described by Francois-Frank[7] and Jackson,[8] but chiefly by a water manometer. The arterial cannula was inserted either into the left pulmonary artery or its branch to the upper lobe. Carotid pressure was recorded by the usual mercury manometer. Experiments were made with open and closed chests, but chiefly upon the former.

Several brands of nitroglycerin tablets were purchased upon the open market. The tablets were dissolved in 1-3 c.c. of saline and injected into a femoral vein. The dosage varied from 0.0006 to 0.006 gm.

Nitroglycerin in doses of 0.0006-0.002 gm. usually caused a transient rise of pulmonary pressure varying from 1 to 60 mm. H_2O. The average elevation was about 25 mm. H_2O in the open chest. (See Fig. 1.†) If the pulmonary pressure was normally high (320-500 mm. H_2O), nitroglycerin lowered it as well as carotid pressure. (See Fig. 3.)

That the rise of pulmonary pressure following nitroglycerin is due to active constriction of the pulmonary vessels is, however, questionable. Indeed,

it seems probable that constriction is not concerned in the action. The chief factors in causing the elevation of pulmonary pressure are: (1) an increase of heart rate due to the effect of lowered systemic blood pressure upon vagal tone, and (2) a transfer of blood from the arterial to the venous system; thus an increase of minute output. For (1) the rise of pulmonary pressure follows from three to ten heart beats after the carotid pressure begins to fall. (2) The height of the pulmonary elevation corresponds to about the low level of the carotid pressure. (3) The pulmonary and carotid pressure curves are roughly diametrically opposed in direction. (4) When cardiac action is impaired by larger doses such as 0.003-0.006 gm., the pulmonary pressure usually falls. (5) The stroke of the ventricular volume curve (as determined by the rubber dam cardiometer) is more complete, and the volume of the heart is diminished during the rise of pulmonary pressure. (6) The increase of pulmonary pressure is dependent to a large extent upon the increase of heart rate. For (a) the increase of rate is more effective if the heart rate was normally slow (below 100). (b) Cutting the vagi when in good tone causes a rise of both carotid and pulmonary pressures. (c) If the medulla is depressed by anesthesia so the heart rate is 180-210, nitroglycerin may cause a very slight rise, no effect or even a fall of pulmonary pressure, whereas the carotid falls distinctly. (d) When the vagi, which have been functioning effectively, are severed, the elevations of pulmonary pressure upon subsequent injections of nitroglycerin are usually diminished and sometimes abolished. (7) In a hypodynamic circulation with vagi not functioning (long anesthesia and exposure or after repeated doses), nitroglycerin produces a fall of pulmonary as well as carotid pressure, whereas epinephrin is still effective in elevating both.

If the pulmonary pressure is normally high, nitroglycerin even in doses as small as 0.5 mg. lowers pulmonary as well as carotid pressures. (See Fig. 3.) Thus it appears that when the pulmonary vessels are under a certain tension, nitroglycerin reduces the resistance in the pulmonary as well as the systemic circuit.

If, with a high pulmonary pressure which falls after nitroglycerin, pituitrin be given, the pulmonary pressure falls much farther from a reduction of cardiac output. If nitroglycerin is now repeated, while the carotid pressure is still high and the pulmonary pressure is low, pulmonary pressure rises temporarily. (See Figs. 3 and 4.)

The importance of the transfer of blood from arterial to the venous system in increasing pulmonary pressure may be seen by connecting the femoral artery to the femoral vein by a cannula. When the arterial flow is released, the pulmonary pressure rises promptly the first or second beat after the carotid begins to fall. The rise of pulmonary pressure is greater in proportion to the fall of carotid than that which occurs after nitroglycerin. (See Fig. 2.)

Nitroglycerin does not stimulate the excised pulmonary arterial rings or strips. This is true also when the tissue is suspended in its own defibrinated blood and the nitroglycerin is dissolved in blood. The doses employed varied from 0.0006 gm. to 0.0084 gm. with the tissue suspended in a 30 c.c. container.

This method for testing excised pulmonary arteries is inadequate to demonstrate depressant action. Thus papaverin, benzyl acetate and benzyl alcohol, as well as nitroglycerin, failed to relax these vessels. The failure to relax is probably due to the fact that the nonmuscular tissues are able to maintain the lifting load. The depressant or toxic action of nitroglycerin can be demonstrated by comparing two tissue suspensions. If one preparation receives nitroglycerin first, it responds very feebly or not at all to subsequent applications of epinephrin or barium, whereas the untreated pulmonary strip contracts vigorously to these drugs.

SUMMARY

Nitroglycerin in small doses often produces a rise of pulmonary pressure due to increased cardiac output from acceleration and increased venous return. Nitroglycerin reduces a high pulmonary pressure apparently by dilating the pulmonary arteries. It does not stimulate excised pulmonary arteries of dogs. Thus the action of nitroglycerin is the same upon pulmonary and systemic arteries, although less marked upon the pulmonary vessels. The action upon pulmonary pressure is, however, often counterbalanced or overbalanced by the secondary effects of its action upon the systemic vessels. The action of nitroglycerin on pulmonary circulation does not justify its administration in the treatment of pulmonary hemorrhage (cf. 1, 2). Experimentally, at least, it appears to be contraindicated in hemoptysis.

REFERENCES

[1]Macht: Jour. Pharm. and Exper. Therap., 1914, vi, 13.
[2]Macht: Jour. Am. Med. Assn., 1914, lxii, 524.
[3]Bradford and Dean: Jour. Physiol., 1894, xvi, 37.
[4]Wood: Am. Jour. Physiol., 1901-1902, vi, 283.
[5]Wood: Jour. Exper. Med., 1911, xiv, 326.
[6]Petitjean: Jour. de physiol. et de path gén., 1908, x, 403.
[7]Francois-Frank: Arch. de Physiol., 1895, p. 744-816.
[8]Jackson and Raap: JOUR. LAB. AND CLIN. MED., 1920, vi, 1.
[9]Wiggers: Am. Jour. Med. Sc., April, 1911.

THE ACTION OF NITRITES UPON PULMONARY CIRCULATION

II. Sodium and Amyl Nitrite*

By George R. Love, M.D., Hugh McGuigan, M.D., and
Charles E. Wiley, B.S., Chicago, Illinois

BRADFORD and Dean,[1] Wood,[2,3] and Petitjean[4] observed that the nitrites increased pulmonary arterial pressure. The former investigators attributed the mechanism to "back pressure" from defective cardiac action, whereas Wood and Petitjean believed that nitrites produced active constriction of the pulmonary vessels. Macht[5,6] reported that the nitrite group (sodium and amyl nitrite, nitroglycerin, and erythrol tetranitrate) stimulated the excised pulmonary arteries of the pig, ox, and human.

Love and McGuigan[7] in a recent communication demonstrated that nitroglycerin under certain conditions produced a rise of pulmonary pressure, due to a greater minute output from cardiac acceleration and increased venous return. If the pulmonary pressure was normally high, nitroglycerin lowered pulmonary as well as carotid pressure We concluded that nitroglycerin has the same depressing action, though less marked, upon the pulmonary arteries as upon the systemic arteries. However, the effects of this action of nitroglycerin upon the pulmonary arteries are often counterbalanced or overbalanced upon pulmonary pressure by the secondary effects of its action upon the systemic vessels.

All experiments were performed upon dogs. The technic employed in this investigation was described in the previous communication.[7]

PULMONARY ARTERIAL PRESSURE

Amyl Nitrite.—The preparation was administered by breaking a five minim pearl in the rubber tubing connecting the tracheal cannula and Woulfe's bottle. The pulmonary pressure in some animals rose from 1 to 60 mm. H_2O following amyl nitrite, while in others the pulmonary pressure fell with the carotid pressure. The rise of pulmonary pressure is not produced by active constriction, but is apparently caused by a greater minute output from cardiac acceleration and increased venous return. Thus, the actions of amyl nitrite and nitroglycerin upon pulmonary circulation are the same, and the arguments presented in the previous paper on nitroglycerin are applicable to amyl nitrite.

Thus, in brief, with a combination of a high carotid, a low or moderate pulmonary pressure, good vagal tone, and a sudden drop of carotid pressure following amyl nitrite or nitroglycerin, the pulmonary pressure rises; whereas, a low carotid, a high or moderate pulmonary pressure, poor vagal tone, and
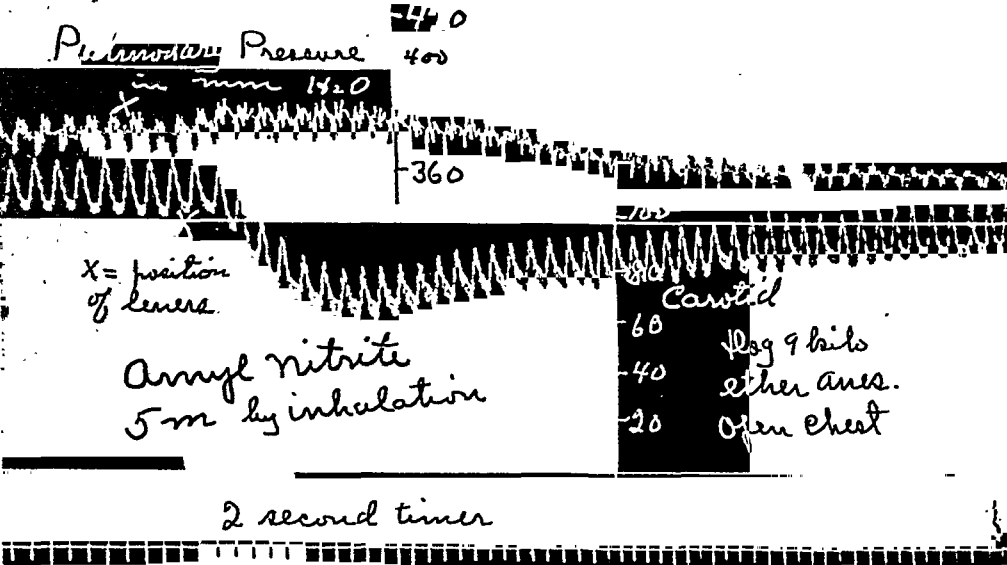
Fig. 1.—A common occurrence after amyl nitrite is a rise of pulmonary pressure, followed by a fall before the carotid pressure returns to normal.
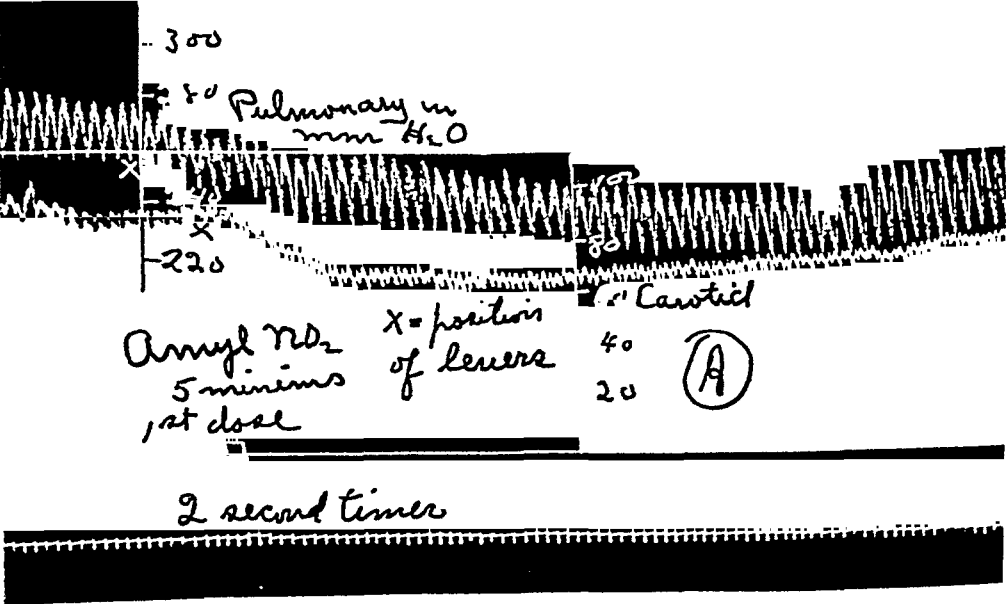
Fig. 2.—First dose of amyl nitrite produced a fall of both pulmonary and carotid pressures. (See Fig. 3.)

a gradual fall of carotid pressure following these drugs favors a fall of pulmonary pressure. (See Figs. 1, 2, and 3.)

Sodium Nitrite.—Sodium nitrite, 10 per cent solution, was injected intravenously in 50, 100, and 200 mg. doses. The smaller doses of sodium nitrite have an action upon pulmonary circulation similar to amyl nitrite and nitroglycerin; however, sodium nitrite produces a rise of pulmonary pressure less frequently than the other nitrites. The frequent failure of sodium nitrite in elevating pulmonary pressure (although amyl nitrite or nitroglycerin may raise the pressure in the same animal) is probably due to the more gradual fall of carotid pressure. (See Fig. 3.) Thus, the cardiac acceleration is less marked, and the compensation for the increased venous return is adequate.

Large doses of sodium nitrite (200 mg.) in a dog under deep anesthesia or after repeated smaller doses of sodium nitrite produce a prolonged fall of both pulmonary and carotid pressures, which is partly due to cardiac depression. In an animal in excellent condition (light ether anesthesia, rapid
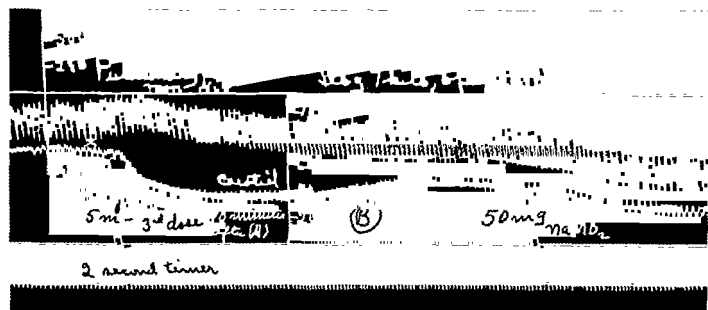
Fig. 3.—Third dose of amyl nitrite causes a rise of pulmonary pressure, followed by a slight fall. Note that carotid pressure falls more rapidly from this dose than in Fig. 2, due perhaps to some difference in preparation or administration.

technical procedures with minimal trauma and hemorrhage), a large single injection of sodium nitrite elicits a pressor action on both pulmonary and carotid pressures. We have made no effort to analyze this pressor action since it does not represent typical nitrite action. That the heart becomes more irritable during the pressor action is evidenced by the frequent appearance of acceleration, extra systoles, and fatal ventricular fibrillation. The action occurs from freshly prepared solutions and also from solutions one year old. We used two different brands of c.p. sodium nitrite (Bakers and Mercks), and dissolved the salts in distilled water.

EXCISED VESSELS

The excised pulmonary arteries of dogs were suspended in Locke's solution at 37° C. in a 30 c.c. container, with a constant stream of oxygen or air. In following the technic described by Macht,[5, 6] we observed no specific action of amyl nitrite or small doses of sodium nitrite on the pulmonary arteries,

but large doses of sodium nitrite (100-200 mg.) caused a powerful contraction of these vessels. This pressor action of sodium nitrite occurs after ergot and atropine. Also, after 1 mg. epinephrin, the addition of 100 mg. of sodium nitrite causes a further contraction of the pulmonary strip, equal to the original epinephrin stimulation. After the sodium nitrite contraction, the tissue, if washed and restretched, will again respond to epinephrin, barium chloride, or sodium nitrite.
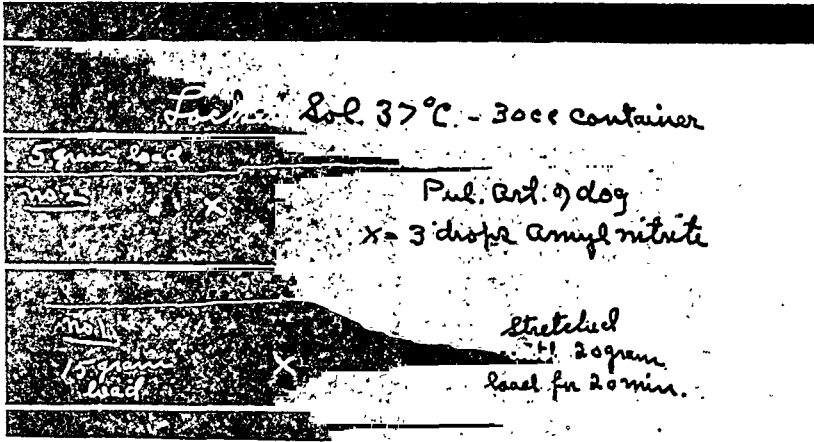
Fig. 4.—The excised vessel was treated as described in text. A 15 gram "relaxing load" was applied, and at No. 1 relaxation had ceased. Note the marked depression following amyl nitrite. The tissue was stretched with a 20 gram load for 20 minutes (without washing out), and at No. 2 the load was reduced to five grams. Note that now amyl nitrite has practically no effect as compared to sodium nitrite in Fig. 5.
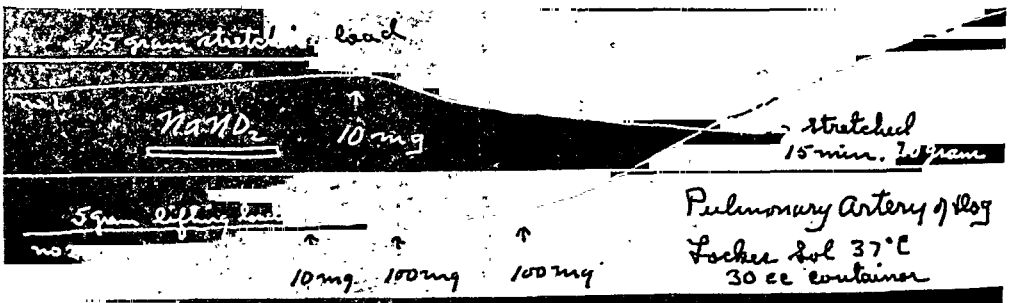
Fig. 5.—In No. 1 the pulmonary strip is relaxed by 10 mg. of sodium nitrite, using the technic described in text. In No. 2 Macht's technic was employed on the same preparation. Note that 10 mg. has no effect now, whereas the larger doses have a well-marked pressor action.

The method employed by Macht consists in stretching the pulmonary strip for fifteen to thirty minutes with a 20 to 30 gram load, then reducing the load to one-fifth or one-sixth of the stretching weights. This technic, as indicated in our previous paper,[7] is not adequate for testing smooth muscle depressants, since after thoroughly stretching the preparation, the extra-muscular tissue apparently will maintain the lifting load. We have modified the technic to demonstrate smooth muscle relaxation as follows: The fresh or ice box pulmonary strip or severed ring is adjusted in the

usual manner in a container (30 c.c. container, Locke's solution 37° C., oxygen or compressed air), and allowed to remain for a few moments without stretching. A load of 10 to 20 grams (depending upon size of the preparation) is applied on the lever while registering on a slow moving drum. After a short period of rapid relaxation, the recording lever usually strikes an horizontal line and sometimes begins to rise very slowly. If papaverine, amyl nitrite, or small doses (5-10 mg.) of sodium nitrite are applied at this time, relaxation occurs. (See Fig. 4.)

We have demonstrated that after a small dose of 10 mg. of sodium nitrite has relaxed the tissue, using the above technic, and the preparation is next weighted to 20 or 25 grams for fifteen to twenty minutes and the load is then reduced to from 5 to 10 grams, the repetition of 10 mg. doses of sodium nitrite produces no effect; but the addition of 100-200 mg. of sodium nitrite produces a marked contraction. (See Fig. 5.)

In perfused pulmonary vessels also, a small dose of sodium nitrite increases, whereas a large dose reduces the outflow. This is illustrated in the following experiment.

Perfusion Experiment No. 7.—Heart and lungs of 8 kilo dog removed under ether anesthesia. Intake cannula inserted through right ventricle into pulmonary artery and tied, outflow cannula inserted into left ventricle through aorta. Locke's solution 37° to 38° C. at intake cannula, pressure kept constant at 350 mm. H_2O. Injections made through T-tube intake cannula. Registration began as soon as heart ceased beating.

Outflow in c.c. per thirty seconds.

20
19
20
20

Injected 25 mg. $NaNO_2$ in 10 per cent solution, washed in with 5 c.c. Locke's solution 37° C.

23
22
19
19
19

Injected 200 mg. $NaNO_2$

27
12
10
6
9
14
18

Amyl nitrite causes an increase flow upon perfusion of the lungs, as may be seen in the following experiment. The amyl nitrite was introduced by breaking a 5 minim pearl in a rubber tubing off the intake T cannula and washing in the amyl nitrite with Locke's solution. (General technic same as preceding experiment.)

Outflow recorded in cubic centimeters per thirty seconds.

25
25
25
24

Injected amyl nitrite

42
33
26
23
23
22
23
24

Our work on excised dog pulmonary arteries may be summarized as follows: Nitroglycerin produces neither relaxation nor contraction with either technic. Amyl nitrite produces relaxation with the method herewith described, and no action with Macht's method. Sodium nitrite in small doses causes relaxation by our method, and no effect by Macht's technic. Large doses of sodium nitrite produce contraction by either method, but is more definite by the technic described by Macht.

DISCUSSION

The hypothesis, that the pressor action of nitrites upon pulmonary arteries is due to stimulation of the constrictor nerves, does not require our particular attention, since we failed to demonstrate a pressor action except in the case of very large doses of sodium nitrite. Since the pressor effect of large doses of sodium nitrite occurs equally well after ergot and atropine, we assume that the action is beyond the neuromuscular mechanism. The fact that large doses of sodium nitrite raise both carotid and pulmonary pressures and frequently cause ventricular fibrillation indicates that the pressor action upon the pulmonary circuit is not specific. The pressor action might be attributed to irritation and not true stimulation. Thus, Douglas[8] observed that a 5 per cent solution of sodium nitrite corroded exposed tissues so as to obscure the peripheral nitrite dilator action. Macht[9] found that sodium nitrite caused a contraction of the ureter, which he attributed to irritation and not to true stimulation. Cook[10] observed that sodium nitrite, when applied directly, increased the rate of the frog's heart.

CONCLUSIONS

Amyl and sodium nitrite, particularly amyl nitrite, often raise the pulmonary pressure in dogs, due to an augmented cardiac output from increased venous return and cardiac acceleration.

Large doses of sodium nitrite often produce a rise of both carotid and pulmonary pressures with cardiac irregularities, which sometime terminate in ventricular fibrillation.

On excised pulmonary arteries, amyl nitrite and small doses of sodium nitrite produce relaxation. Large doses of sodium nitrite cause a contraction of these vessels, which we suggest is caused by irritation and not true stimulation.

The action of amyl and sodium nitrite upon the pulmonary arteries is the same, though less marked, as upon the systemic vessels; however, the effect upon pulmonary pressure is often counterbalanced or overbalanced by the secondary effects of the action upon the systemic circulation.

REFERENCES

[1] Bradford, and Dean: Jour. Physiol., 1894, xvi, 37.
[2] Wood: Am. Jour. Physiol., 1901-1902, vi, 283.
[3] Wood: Jour. Exper. Med., 1911, xiv, 326.
[4] Petitjean: Jour. de Physiol. et de path. gén., 1908, x, 403.
[5] Macht: Jour. Pharmacol. and Exper. Therap., 1914, vi, 13.
[6] Macht: Jour. Am. Med. Assn., 1914, lxii, 524.
[7] Love, and McGuigan: Jour. Lab. and Clin. Med., 1925, x, 882.
[8] Douglas: Jour. Am. Med. Assn., 1924, lxxxii, 381.
[9] Macht: Jour. Pharmacol. and Exper. Therap., 1917, ix, 427.
[10] Cook: Am. Jour. Physiol., 1909, xxiv, 263.

EDUCATION AS A CURE FOR PRESENT-DAY EVILS IN CLINICAL PATHOLOGY*

By John A. Kolmer, M.D., Philadelphia, Pa.

THE past fifteen years has been a period of especially rapid progress and development in clinical pathology, if we employ the term in its broadest sense to include those parts of clinical chemistry, clinical bacteriology and clinical serology constituting the work and scope of the average laboratory engaged in medical laboratory examinations. It is but natural that certain evils have crept into the field, as is always so likely to occur under such circumstances, and we hear much these days of the necessity for correcting them, this Society itself being dedicated to the task as one of its most important national activities. For several years I have been greatly interested in the situation and in a consideration of ways and means for improving the status of clinical pathology as a specialty in medicine, not only as a member of this Society, but likewise as Chairman of a Committee on Laboratories of the Pennsylvania State Medical Society, delegated to investigate conditions in this state and recommend remedial measures. At first I thought that the best way was probably the easiest way, namely, by legislation. But I now believe that legislation alone is probably insufficient, as it is comparatively easy to make laws but more difficult to enforce them, and I have finally reached the conclusion that education in a broad sense is probably one of the best and most reasonable weapons at our command, as has ever been true in the history of human progress. It will be my purpose this evening to define briefly some of the existing conditions which in a broad sense may be regarded as evils in the field of clinical pathology and to suggest how education may be reasonably expected to correct them in whole or in part.

As members of this Society we are dedicated to the principle that medical laboratory diagnosis or clinical pathology in a broad sense, should constitute a specialty in medicine just as surgery, ophthalmology and other clinical subjects are so regarded; but are we educating ourselves to fulfill this destiny? We recognize as an evil the taking over of this work by those who do not have

a medical education, but does the merely technical phase of laboratory work demand such? Is it not true that many clinical pathologists are content to merely do the work which well-trained technicians are capable of conducting in just as acceptable a manner? If such is true we must first recognize the possible existence of an evil in our own ranks because clinical pathology will not readily win for itself the exalted position of a specialty in medicine as long as the subject is held to the merely technical phases of routine medical laboratory examinations. As so well and so frequently stated by our fellow members, Hillkowitz, Burdick, MacCarty and others, we must prepare ourselves to know disease in its broadest aspects and to serve our fellow practitioners as consultants in this field; we interpret the term to include, therefore, not only the training and experience necessary for the proper interpretation of routine examinations but the willingness to apply special measures for aid in the diagnosis of particularly difficult cases and to offer or even outline biologic and chemotherapeutic measures in treatment. In other words, merely to make routine laboratory examinations which a well-trained nonmedical technician may make just as well is not sufficient for elevating clinical pathology to a specialty in medicine, and the attitude of mind and practice that regards such to be the case is to be looked upon as an evil of first rate importance. If we insist upon a medical education as a requisite for the recognition of the clinical pathologist as a specialist in medicine, it must be under such circumstances as will draw upon medical education and experience; he or she must be willing and prepared by education to assume more responsibility in diagnosis than the technician is capable of assuming, and it is especially necessary to keep abreast of all advances in this field in order to be ever ready and willing for trying new methods of promise, possibly to conduct some original research, to keep in touch with clinical medicine and surgery and, lastly, as previously stated, to be prepared to advise in treatment and especially in the treatment of infectious diseases by biologic and chemotherapeutic agents.

Upon us falls the responsibility of proving that clinical pathology is a specialty in medicine deserving of wider recognition and adequate compensation. Just as the physician cannot hope to be successful by merely doing the work of a well-trained nurse, or the roentgenologist hope to establish himself as a specialist by merely doing the work of a technician in this field, so likewise the clinical pathologist cannot hope to qualify as a specialist by merely conducting routine laboratory tests readily handled by nonmedical technicians. He must be prepared by education and experience to do more, to shoulder more responsibility when occasions arise and by these means prove that clinical pathology in its broad sense is indeed a real and important specialty in modern medicine, full of opportunities and promising in financial returns, rather than a good camping ground for the misfits and all and sundry who choose to enter the field.

That we have a real need for the laboratory technician cannot be denied. Indeed, I believe the well-trained technician is almost as indispensable in the laboratory as the nurse at the bedside. I am sure that a hospital or a community without a good pathologist but with a good laboratory technician is

much better off than a community or hospital without either. But unfortunately the true functions of the technician are not as widely and clearly understood as they should be and the tendency for them to actually practice medicine has amounted in some localities to an evil requiring adequate measures for correction. There is today a wide field of usefulness for well-trained technicians, not only as assistants to practitioners of medicine too busy to conduct their own laboratory examinations, but likewise as assistants to pathologists whose time can or should be far more profitably spent than doing the simple routine laboratory tests. Naturally a large number of practitioners wish merely to hire someone to conduct the tests, while leaving the matter of interpretation of results for themselves. And as long as this is actually the case I can see no harm in the situation, but not infrequently the technician assumes, or has forced upon him or her, the latter function which may automatically make them diagnosticians and thereby in a certain sense practitioners of medicine, since their information not infrequently reaches the laity direct. How to correct this situation is one of the most perplexing questions of the present day. The remedial measures must not deny the physician or institution the right to employ the technician. Legislation may readily define the limits of their activity and forbid the nonmedical individual from infringement upon the practice of medicine but it is one thing to spread laws on our books and another to enforce them; furthermore, our state boards of medical licensure have their hands full in dealing with far more dangerous and pernicious infringers of the various medical practice acts than the laboratory technicians. In the correction of this evil I believe that education is particularly full of promise, and I refer especially to the education of the practitioner of medicine, secondarily to that of the laity and lastly to that of the technician.

I believe it may be stated without reserve that if the medical profession so will it, every irregular and incompetent laboratory and technician could be put out of business by a lack of patronage. It is true that some commercial laboratories flourish by the patronage of the laity but in a general way they confine their activities to the examination of the urine and it is to be hoped that in time the laity could be educated to an appreciation of their very limited value and quackish character.

But there is a real need for the education of the medical profession in two main directions, namely, the education of those physicians who employ the laboratory to exercise more care and discretion in the choice of the laboratory to which they entrust their work and, secondly, the encouragement by education of those physicians who do not use the laboratory at all or but seldom, to a better realization of the aids in diagnosis and treatment offered by the well-trained pathologist and adequately equipped laboratory. If physicians could be more broadly educated to look upon clinical pathology as a specialty in itself through the efforts of clinical pathologists to make it so, and to refer their work to medically trained pathologists, the field would be more rapidly developed, more rapidly placed on a satisfactory basis for clinician, pathologist, and layman alike and render the specialty of clinical pathology more attractive to the medical man or woman as a field for practice.

Indeed I doubt if anything more need be done to correct the existing evils, and this Society, in cooperation with other national societies, may do well to launch such an educational campaign through the state and county medical societies, while establishing at the same time standard qualifications for both pathologist and laboratory and aiding institutions and communities ·in securing both.

It is certain that at the present time there is not a sufficient number of qualified pathologists to supply the needs of the country at large; it is also certain that the supply is insufficient because the specialty has not been made sufficiently attractive for graduates in medicine and, finally, it is certain that unqualified individuals have stepped into the breach to supply the demand. A pernicious cycle must be broken and in my opinion it can be best broken by a well-directed educational campaign. I am convinced that many more of our young graduates in medicine would take up clinical pathology as a specialty if it were made more attractive; I myself cannot encourage any young medical man to enter this field merely as a competitor of the laboratory technician with the comparatively low professional and financial returns, but the situation could be changed and the field rendered increasingly attractive by better cooperation and the exercise of better judgment on the part of practitioners of medicine and surgery and these results are to be obtained only by education of the latter. One is tempted to state that this education should begin in our undergraduate medical schools but our educators and students alike are so harassed by the multitude of subjects to be taught and learned that one hesitates to add to their burdens, although it is to be stated that many of our medical schools could greatly improve their teaching in this field by providing better facilities for the teaching of clinical pathology during the third and fourth years; but of far more importance than the teaching of mere technic is the matter of the teaching of the broad and very practical applications of clinical pathology in the diagnosis and treatment of disease and the necessity for having the work done well if it is to be done at all. In a general way, however, we see the results of such teaching at present among our younger practitioners; it is among the older ones, those who did not have such opportunities for instruction in their undergraduate days, that we see most need for education in these matters. This education should consist in the proper evaluation of laboratory examinations as a safeguard against depending upon them either too much or too little and to show physicians that the work is best done and that they will be best helped by calling upon the medically trained clinical pathologist to at least supervise such examinations and to serve as a consultant should the case require expert knowledge in this particular field.

I may pass over even more hurriedly the subject of education of the laity except to state that education of the medical profession should be first, since there is no better educator of the layman than his medical adviser. But in addition, a great deal may be done by a well considered educational campaign through such publications as *Hygeia*, the magazine of health published by the American Medical Association, by radio talks and even newspaper articles prepared and disseminated by such responsible medical associations as our

county and city medical societies. Indeed, I am inclined to believe that the laity is following us so closely in all that pertains to the eradication of the unworthy in medicine, that they may furnish some of the stimulus and incentive to the medical profession for cleaning house in matters pertaining to the diagnostic laboratory.

At any rate I believe the time is ripe for a wider recognition of existing evils and an earnest consideration of education along these various lines for their eradication. I have the impression that we are only waiting for leaders to appear with definite ideas and definite plans, and I believe that the American Society of Clinical Pathologists is the logical body to undertake the task, working in heartiest cooperation with any other national or local society imbued with the same ideals and purposes. Education is slower than legislation but it is the surer and better way; this Society has already made a good start and the task may not be as great when actually under way as we view it now from a distance.

At the present time our attention is commanded by discussions and experiments in the standardization of laboratories since so much has been accomplished by the American College of Surgeons in the standardization of certain hospital activities. But I am sure that the matter of standardization of laboratories arouses in others the same conflict of opinions and emotions as it does in me. It is right, proper and helpful for the State to insist upon the laboratories of institutions receiving state aid to possess a minimum of equipment and an adequate personnel since the public funds are being expended; likewise in the case of those laboratories to be recognized as affiliated with the state laboratory for examinations in the infectious diseases after the plan developed by Wadsworth and Gilbert in New York. But the State has no more right to inspect or meddle with the work of the private laboratory of the duly examined and licensed medical graduate practicing clinical pathology than it has with the office and practice of any other physician, and due care in this direction must be exercised against socializing medicine. I am heartily in favor of empowering our State Boards of Medical Licensure to exercise a more adequate control over laboratories being conducted by non-medical individuals because the health of individuals and even of the public may be and is now being jeopardized by incompetents in this field, but I believe that most will be accomplished in time by our Society establishing minimum qualifications for the specialist in clinical pathology, providing for an examining board and having her certificate recognized by an educational campaign as the badge of approval on the clinical pathologist bestowed by those in best position to judge of the qualifications and equipment of the applicant, for the guidance of institutions, the medical profession and even the laity seeking the services of a real specialist in this field of practice.

And while we are considering the State in relation to the correction of existing evils in laboratory medicine, may I suggest that the present activities of many state and municipal laboratories are so rapidly socializing this field of practice as to constitute a growing evil? No one will deny that these laboratories have done and are doing a great service in relation to the diagnosis and control of the infectious diseases and there is a real need for them in this

field of work since the public is involved, but the present tendency for doing all kinds of laboratory work free of charge and making the most strenuous efforts to obtain it for presenting statistical evidence of the growing popularity and activities of the laboratory or its director to all who may be subject to impression by such should be curbed as a growing evil, unless we entertain the opinion that medicine should be socialized and that here is the best possible start toward this end.

Before concluding these remarks I wish briefly to refer to two other educational matters in relation to clinical pathology, namely, the education of the technician and of the hospital interne, since these are fields in which we may all contribute a share. As previously stated I believe the well-trained laboratory technician is a blessing and indispensable for the proper conduction of the medical laboratory. But let us better realize our responsibility in his or her training, aiming to teach them good and acceptable methods, the necessity for hard and conscientious work and that there is no place for the careless worker interested alone in the financial returns or for the "sink-test specialist." In the final analysis the majority learn by observing the way we do things, and it is our duty to clearly define for them what they may readily learn to do well and acceptably and that, beyond the purely technical phases, a broad and comprehensive medical education becomes increasingly valuable. I am convinced that laboratory technicians are yielding on the whole valuable service to the medical profession and there is such an increasing demand for their services, and especially as assistants in institutional laboratories, that the situation may well command the attention of this Society as an additional field for service.

For example, I should like to see our Society look into the matter of present-day methods for educating technicians and to establish minimum standards; also to establish a bureau of examination or at least of registration to which physicians and institutions may look for aid in obtaining the services of a qualified technician. In this connection I may state that the training of technicians has commanded my attention for the past eleven years and the system now in vogue in the laboratories of the Graduate School of Medicine of the University of Pennsylvania is proving very successful and may be readily adopted by any large laboratory. In the former Polyclinic and College for Graduates in Medicine I gave a formal course of instruction in clinical pathology, clinical bacteriology and clinical serology embracing lectures and practical laboratory work. But when this institution became amalgamated a few years ago with the University of Pennsylvania, the dean of the Graduate School of Medicine, Dr. George H. Meeker, decided that these courses should be dropped in order to permit us to better take care of the increasing numbers of student graduate physicians. Realizing that the training of technicians was rendering a distinct service I then proposed, and the Dean promptly accepted and endorsed, the present system of accepting a few students each year under a volunteer assistantship plan by which the applicant agrees to serve in the laboratories on a full time basis for a period of sixteen months. This time is divided into four periods of four months each in the laboratories of histologic technic, clinical pathology and bacteriology, blood chemistry

and serology. Each laboratory is in charge of a highly trained assistant under whom the volunteer works and learns by actually assisting in the work of the institution from the very first to the very last day of the course. The work is entirely practical and technical, supplemented by evening reading and study. The University now requires each appointee to make a deposit of $160 but this is returned at the rate of $10 per month, making the course entirely free of tuition. The plan has worked admirably and the technicians are certainly acquiring a much better training and have a much better understanding of their real capacities and functions than was possible under the former plan. It is true that they require instruction and that the plan is bound to fail unless the instructors are in sympathy with the idea, but the volunteers soon learn to become useful and the plan is truly and indeed upon a fair and just *quid pro quo* basis. I am hoping very much that other laboratories will take up the plan which is after all nothing more than a refinement of the oldest plan of all, namely, the apprentice system, and I am in position to state that the laboratory is able to conduct a much larger volume of work without an increase of the salary budget than is otherwise possible, while a distinct and valuable service is being rendered by furnishing a source of supply of fairly well trained experienced laboratory technicians who are just technicians without fake notions of being pathologists or physicians.

In conclusion I wish to enter a plea for more care in the education of the hospital interne entrusted to us for training and experience in the laboratory. Here is a golden opportunity for education along the lines so necessary in my opinion for the advancement of the cause of clinical pathology. Too frequently the interne is allowed to do much as he pleases or is required to spend his valuable time in the simplest kind of laboratory work like urinalyses and blood examinations. No wonder that under such conditions the majority of internes look upon the laboratory service as something to be endured rather than an opportunity to be welcomed. It is true that their function is to serve the institution but it is equally true that they have every reason and right to expect instruction and the opportunity to learn and I would suggest that we embrace this opportunity for educating these young men and women into a proper appreciation of laboratory medicine by giving them more of our time and instruction, more opportunities for assisting in a variety of laboratory examinations of all kinds instead of tying them down to a fixed and monotonous routine which turns them out with the idea that clinical pathology is merely a field for the nonmedical technician, instead of warranting their serious consideration as a specialty in medicine well deserving of their closest attention and study.

THE RÔLE OF THE CLINICAL PATHOLOGIST IN HOSPITAL EFFICIENCY*

By Malcolm T. MacEachern,† M.D., C.M., Chicago

I HAVE selected this subject for my remarks because I am fully convinced that the clinical pathologist plays an important rôle in hospital efficiency. It, therefore, affords me much pleasure to discuss this subject with you for a few minutes.

The American College of Surgeons ten years ago formulated a minimum standard of requirements for hospitals. This standard consists of broad, adaptable and adjustable principles. One of these principles is of interest to the American Society of Clinical Pathologists inasmuch as it concerns clinical laboratory service in hospitals. I refer to Clause 5 of the Minimum Standard, which reads as follows:

> "That diagnostic and therapeutic facilities under competent supervision be available for the study, diagnosis and treatment of patients, these to include, at least (a) a clinical laboratory providing chemical, bacteriological, serological and pathological services; (b) an x-ray department providing radiographic and fluoroscopic services."

The above requirement presupposes the following considerations:

I. ADEQUATE ACCOMMODATION

Proper spacing, planning, airing, lighting and accessibility are desirable for the clinical laboratory. Fortunately, in recent years, there is a tendency to depart from the custom of placing this department in the basement. It is generally agreed today that the clinical laboratory deserves as desirable a location as any other department in the hospital. Personally, I have been advocating its location in or adjacent to the operating room suite. The best location, however, can be selected by determining where contact will be afforded with the greatest number of the medical profession at all times.

There has been too much isolation of this department in hospitals in the past. The old saying, "Out of sight, out of mind," applies in this case. Isolation tends to minimize use and service to the profession. No doubt that with the rapidly growing importance and increased use of the clinical laboratory, more attention will be directed towards the better locating of this department.

It is also desirable, where possible, to make the necessary physical divisions as to table and wall space for the various activities carried on, namely, chemical examinations, clinical microscopy, bacteriology, serology, pathology and blood chemistry. This tends to more efficient operation.

A number of the larger hospitals today find it advantageous to have one or more smaller working laboratories scattered throughout the wards and subsidiary to the main department. Frequently we find the tissue laboratory placed in or adjacent to the operating room suite, so as to be more readily accessible for gross and microscopic examination of all tissues removed at operation. An arrangement of this kind has three distinct advantages: (*a*) It minimizes errors in identification of specimens. (*b*) It tends to better preservation of natural appearances and qualities of the specimens removed by shortening the time of transportation. (*c*) It affords closer association between the surgeon and the pathologist. This arrangement is always commendable.

Ward laboratories are of distinct advantage to the clinician and the interne, providing they are under proper supervision. Personally, I favor the consolidated laboratory system where the facilities, technic and personnel can be kept under constant proper supervision. I would, however, make one exception to this, as mentioned above, that is, the having of the tissue laboratory in or adjacent to the operating room suite if the whole department cannot be so located. I believe that too many small laboratories scattered throughout the institution renders proper supervision of equipment, technic and workers impossible, and, therefore, inaccuracies in technic and interpretation may result. We all realize full well that bad laboratory work is worse than none at all.

II ESSENTIAL FACILITIES

It is generally agreed upon that there are five branches of work carried on as a minimum in the average laboratory today. These are: (*a*) Chemistry or Clinical Microscopy, (*b*) Bacteriology, (*c*) Serology, (*d*) Pathology, (*e*) Blood Chemistry. Every hospital requires these services in order to do proper work and discharge its full responsibility in the best scientific care of the patient. Laboratory equipment is today fairly well standardized and, therefore, I need not dwell on this phase of the subject.

In passing, may I suggest that I believe we must look to this society for standards in planning, equipment, personnel and technical procedures? There is a continuous demand from the field in this respect, and authorized information from such a body as yours would be greatly appreciated and of value to the hospital field. It is necessary today that the hospitals be provided with carefully worked out minimum standards for the various activities carried on. Given these standards they will build up the service more efficiently in approximating a maximum.

III. EFFICIENT PERSONNEL

A fine clinical laboratory with excellent appointments and facilities does not always mean efficiency. This, of course, is desirable, but it is the personnel of the department which counts most. This personnel generally falls into three divisions:

1. *Supervising or Directing.*—The supervising and directing of the work of the clinical laboratory can only be done efficiently through a well-trained,

competent clinical pathologist, part or full time, depending on the nature and amount of work to be carried on. It is unfair to throw responsibility of laboratory work on the technician, both from the standpoint of complicated technic and interpretations.

While most hospitals today have satisfactory technical services the American College of Surgeons believes and requires that the clinical laboratory be under the supervision and direction of a competent clinical pathologist. A fundamental medical knowledge with subsequent special training and experience is essential for proper and accurate interpretation of findings, as well as for the development of technic.

We are informed on good authority that there are between seven and eight hundred clinical pathologists available in the United States and Canada, to take care of the work in some seven or eight thousand hospitals, of which approximately twenty-five hundred are active general hospitals of thirty-five beds and over. It is, therefore, quite evident that there is distinct shortage of well-trained clinical pathologists. The best solution for this problem at present is the grouping of hospitals under one clinical pathologist, providing sufficient technical service in each institution to do the work efficiently.

2. *Technical Service.*—Technicians render most excellent service under competent supervision. This work, I find, is usually taken up by nurses and university graduates. The nurse technician in the smaller hospital, where her full time is not required in the laboratory, is able to assist with other work. Not infrequently do we find many combinations of services effected, as for instance: (*a*) Laboratory and various types of nursing or administrative activities in the hospital; (*b*) Laboratory and x-ray technic; (*c*) Laboratory and case records; (*d*) Laboratory and x-ray technic and case records. However, in all these combinations let us avoid placing too many duties on the technician. In most instances a technician can be kept busy in any one of the above mentioned services.

I cannot dismiss this part of my discussion without emphasizing the importance of having properly trained technicians. Many instances are known where laboratory errors have been most embarrassing, not to say anything of the damage they may have done to the patient. Let us not forget that untrained and improperly supervised technicians are very dangerous in any institution.

3. *Janitor Service.*—Every laboratory requires competent janitor service to keep the department clean and orderly. Not infrequently we find this feature overlooked, to the detriment of the worker and the efficiency of the department. A good janitor service will embrace: (*a*) Keeping the room and equipment clean and orderly. (*b*) The proper storage and care of bodies. (*c*) The feeding and care of the animals used for laboratory purposes. (*d*) The assisting with certain procedures as required. Many other duties are generally attached to this service.

IV. PROPER ORGANIZATION

The clinical laboratory is one of the important service departments or units in the entire organization of the hospital. It is one of the contributing

units in service to the patient and comes under the medical division, taking its place both in the diagnostic and therapeutic aspects of medicine. It must be thoroughly organized. As already stated, a department head or director is necessary, who shall be responsible to the hospital management for the operation of this particular department. Assistants, technical staff and others should be directly responsible to the head of the department.

The entire staff must be imbued with earnestness in their work and the doing of everything to render the best service possible to the patient. They must also be imbued with the spirit of cooperation, coordination, efficiency and economy in the management and operation of the department. Underlying the technical service there must be a distinct personal interest in the department itself as a unit of the larger or more complete organization of the hospital.

V. LABORATORY RECORDS

A good record system in the hospital today consists of many units contributed by the various departments concerned. One of these units is that of the clinical laboratory, which should establish and carry out a definite, simple but thorough record system. The following outline may be considered as a basis for such a system:

1. *Requisition for Work Desired.*—This form should be applicable for all examinations. Ample space to give a very brief abstract of clinical data is desirable. Too many requisitions come to the laboratory with indefinite requests and information, thus placing the clinical pathologist at a distinct disadvantage. A comprehensive requisition method will not only expedite matters but will assist the clinical pathologist in giving a more intelligent service.

2. *Report of Findings.*—This requires to be made in duplicate, one copy to go to the patient's file and the other to remain in the department as part of the record system.

3. *Daily Report.*—The daily report shows the various consecutive examinations made each day. This may be recorded in a loose leaf system.

4. *Filing of Reports.*—Usually the vertical folder and cabinet is used for the filing of the report, arranged alphabetically or according to the patient's number.

5. *Cross Index.*—Many methods of cross indexing laboratory reports are found. A good system makes it possible to find data by name, hospital number, and at the same time provide the necessary grouping of the various types of examinations and findings.

Frequently it is found preferable to assign a laboratory number to each requisition. If this is done it must not be so placed as to confuse it with the hospital number, which should be carried throughout. Requisitions may be filed under the doctor's name. In this way the amount of laboratory work called for by each doctor is readily checked up.

A summary laboratory sheet has been found desirable and practical in many hospitals. This sheet immediately affords the doctor in charge of the patient a bird's-eye view of the amount of laboratory work done over any given period and saves him from searching through many forms.

VI. LABORATORY CHARGES

A great deal has been said and written during recent years in regard to laboratory charges. However, it still remains a matter of much diversified opinion as to the best methods for a hospital to adopt. A review of the situation indicated the following methods in use at present:

1. Adoption of a schedule of charges for the various tests performed, varying according to the nature of the examination.

2. Including of this service in the *per diem* rate charged to the patient.

3. Adoption of a flat rate to include all the laboratory work.

4. Providing a flat rate for some of the routine work, and making a charge for all other examinations required.

5. Providing a free service, as might be granted through the federal, state, county or endowed laboratory.

6. Turning of the work over to a clinical pathologist who secures part or all of the fees and provides the necessary free service.

The American College of Surgeons has not recommended any particular system, but urges that whatever method is used it should not limit or embarrass the amount of work called for and required in the best interests of the patient. It is hoped, however, that the near future will see a more uniform policy of charges worked out for hospitals.

VII. ROUTINE EXAMINATIONS

There is also much diversified opinion as to what examination should be routinely carried out in the hospital today. The following are generally given the most consideration in this respect: (*a*) Urinalysis; (*b*) Blood examinations, red, white and hemoglobin; (*c*) Blood coagulation; (*d*) Wassermanns; (*e*) Tissues from operation. The American College of Surgeons has left this matter to each hospital to work out, with the exception that they insist on the routine urinalysis and tissue examinations. Considerable variance of opinion exists as to whether tonsils should be examined pathologically or not. However, I am pleased to state that each year more and more hospitals are including the pathologic examination of tonsil material in their routine work.

VIII. CLOSE CONTACT BETWEEN THE CLINICAL PATHOLOGIST AND THE MEDICAL STAFF

It is desirable that the closest contact be maintained between the clinical pathologist and the medical staff. In the past this has not always been the case. The clinician and the clinical pathologist must work hand in hand if we are going to get the best results. This can be brought about in several ways:

1. Placing of the laboratory in a location accessible to the members of the medical staff, where contact is much more frequent. The laboratory should be open to the clinician at any time he wishes, and he should always receive as much personal attention as can be afforded him.

2. Placing of the tissue laboratory in or adjacent to the operating room suite will bring the surgeon and the clinical pathologist into much closer contact. The clinical pathologist should be available for the surgeon when he is operating, if he requires him. It may be an advantage to the surgeon to have

the clinical pathologist see the tumor before it is removed and advise with him in regard to its removal. It is advantageous to the surgeon to have the clinical pathologist demonstrate the freshly removed tumor to him immediately after operation. In this way the surgeon keeps abreast with his gross pathology. In addition, the mutual study of the gross specimen in a fresh state will usually prevent difference of opinion arising between the surgeon and pathologist later as to diagnosis. Again, the surgeon may follow the section through the laboratory and study the microscopic findings. There is no doubt in the world but what the surgeon becomes a better surgeon from contact with the clinical pathologist, and the clinical pathologist becomes a better pathologist from his contact with the surgeon. Therefore, let us stimulate this teamwork in hospitals as much as we can.

3. The clinicopathologic conferences now held in all well-regulated hospitals are of untold value in bringing the clinical pathologist and the medical staff closer together. They have made staff conferences more interesting. The findings of pathologic conditions, ante- and postmortem, in themselves cannot be of much worth unless made use of and studied in relation to the living and normal. The clinicopathologic conference should embrace gross and microscopic pathology. The subject matter of such a conference may be presented under four headings:

(a) History of the case. (b) Demonstration of gross and microscopic findings, either through the actual specimen or lantern slides. (c) General discussion of findings in relation to history. (d) Conclusions to be drawn from the study of this material.

A careful record of the clinicopathologic conference should be kept and the discussion and findings attached to the respective case histories discussed.

The clinical pathologist must not be regarded in future as a mere technician to work in his laboratory and not mingle with the other members of the profession. More and more he is being called in by the clinician as a consultant at the bedside of the patient. Not infrequently the clinical pathologist may render the clinician material assistance in determining new lines of procedure as to diagnosis and treatment. And this is as it should be, for the fundamental principle of hospital standardization as advocated by the American College of Surgeons is the surrounding of the doctor or clinician, be he surgeon, physician or specialist, with all that is available in medical science in order that he may give his patient the maximum service in diagnosis, treatment and end-results obtained. Unfortunately, in a few instances, there is a feeling of lack of confidence in the clinical pathologist by the surgeon, who is sometimes skeptical as to the value of the pathologic service to the hospital. This can be remedied only through a working contact as I have endeavored to outline.

IX. PERIODIC CHECK-UP ON WORK AS TO QUANTITY AND QUALITY

The clinical pathologist must constantly check up on the laboratory service as to quantity and quality. A hospital may be doing too much or too little laboratory work for the patient. This can be determined from a study of each case, and especially should be noted through the staff conference

analysis. The clinical pathologist must always keep a watchful eye over the quality of the work. In checking this up he should have access to the patient in order to compare clinical findings with laboratory findings. In examining a hospital some time ago I found they had a wonderful laboratory, excellent equipment and splendid personnel. Out of the entire volume of work done during the year there were only six tissues examined, notwithstanding the fact that there were fourteen hundred and twenty-six operations, with approximately seven hundred showing removal of tissue in one form or the other. While this hospital was doing very creditable laboratory work, it seems to me they failed to carry out one of the essential services. The American College of Surgeons has found it necessary to check up the quantity and quality of laboratory service in all hospitals surveyed. It is difficult to determine the quality of laboratory service, but comparatively easy to ascertain the quantity. The monthly analysis sheet, which we require each hospital to fill in, contains on the reverse side a detailed report of the various laboratory examinations made each month.

X. DIFFICULTIES ENCOUNTERED IN THE SMALLER HOSPITALS

We have met with difficulty in establishing efficient laboratory service in the smaller hospitals, particularly in getting this work under competent supervision. It is not difficult to maintain a good technical service in these institutions. Frequently part of the service can be secured from a neighboring institution or through the federal, state or municipal laboratory. However, we insist that every hospital makes provision for the emergency examinations that are necessary. By this I mean such examinations as are essential in assisting the doctor to make his diagnosis as quickly as possible. The American College of Surgeons believes that every hospital should be able to do the following tests without delay: urinalysis, blood examinations, coagulation, smears and spinal fluid. Serology, pathology and some of the other tests can be sent out for report. The American College of Surgeons, in its hospital standardization program, has adopted the rule that necessary laboratory service should be available without delay, regardless of where it is to be secured from.

Supervision of the smaller laboratory has been difficult. In many instances a clinical pathologist is not available for either part time or full time duty. Occasionally one of the medical staff now in clinical work, but who has had previous pathologic training and experience, is secured to assist in supervising the department until better arrangements can be made. The grouping of hospitals under one clinical pathologist, with technical service in each, has helped considerably to solve this problem.

XI. CONCLUSION

I cannot conclude this paper without making reference to the great increase in hospital efficiency which has resulted through the establishment and development of the clinical laboratory during the past few years. I believe that the clinical laboratory has done much to improve medical service, especially in the following respects:

1. The assisting of the clinician in making a more accurate and intelligent diagnosis. The clinician today uses the clinical laboratory in numerous ways

to assist him in making or confirming diagnoses. The many available examinations have made diagnoses more accurate, as well as shortening the time in working them out.

2. The eliminating of unnecessary, incompetent and illegal surgery. No longer do we find the specimen from operation thrown away in the bucket. Now, in properly regulated hospitals, it is handed over to a competent, well-trained clinical pathologist who analyzes it carefully and describes it macroscopically and microscopically. These recorded findings are thoroughly reviewed by the staff of the hospital and thus incompetent, unnecessary and illegal surgery more readily ascertained.

3. The stimulating of a more thorough clinical interest on the part of the staff through the clinicopathologic conference. The study and demonstration of gross and microscopic tissues in relation to the clinical findings tend to vitalize staff conferences and increase much greater interest.

All the above has contributed much towards improving medical service in hospitals. The American Society of Clinical Pathologists has done a great deal towards promoting the cause of better laboratory service in hospitals. Its assistance is still very much needed. Being deeply interested in hospitals and the improvement of service, I am almost bold enough to lay out a program for your society which I am sure would do much to elevate our hospitals to a higher plane of efficiency. Personally, I feel that there are five problems to which this society should give attention. Perhaps it has done so already. Here are my suggestions:

1. The stimulating of the training of more clinical pathologists.

2. The standardizing of the training of clinical pathologists and technicians.

3. The standardizing of planning equipment and facilities for laboratory work.

4. The making of a survey of the United States and Canada for the purpose of determining the number of available clinical pathologists and their credentials.

5. The preparing of a list of text and reference books and magazines suitable for medical libraries in hospitals.

In conclusion, let me thank you for your support in our hospital standardization movement which today embraces over twenty-four hundred hospitals in the United States, Canada and other countries. Again let me call your attention to the fact that this movement is based entirely on service to the patient, and taking into consideration all the contributing units, the clinical laboratory with its service is one of the most important.

A STUDY OF TISSUE AUTOLYSIS IN VIVO*

II. A Pharmacological Study of the Toxic Material

By Edward C. Mason, M.D., and Edward C. Davidson, M.D.

Assisted by P. B. Rastello

IN a previous paper on this subject[1] we have called attention to the fact that the autolysis of a small amount of liver tissue free within the abdomen is accompanied by marked and constant changes in the animal's blood chemistry. We also expressed the opinion that such chemical changes did not give an adequate explanation as to the cause of death accompanying the autolysis.

The present study was undertaken with the hope of gaining more definite information as to the (1) nature of the toxic material, (2) its degree of toxicity and (3) its mode of action. During the study, observations were made using (1) extract of fresh ground liver, (2) extract of autolyzed liver, (3) abdominal fluid present at the time of death accompanying autolysis, and (4) nonprotein fraction of the autolyzed liver extract. Since completing the experimental portion of this work it has been our pleasure to read the splendid report of Cannon[2] on the "Evidence of a toxic factor in wound shock." Although we have approached the problem from an entirely different angle there appear to be many points in our observation which are subject to interpretation in terms of Cannon's report. He has made the distinction between *primary* and *secondary* shock, and it is especially to the latter which we wish to refer. In the study of "wound shock" Cannon describes his experiment as follows: "In order to bring about, in lower animals, a traumatization similar to that giving rise to shock in man, the thigh muscles in the anesthetized cat, while being supported by an iron block, were repeatedly struck with a blunt wedge-shaped hammer, or they were crushed by compression. The trauma usually failed to break the skin, so that infection from without was impossible." After about twenty minutes the blood pressure of such animals began to fall, and after about one hour it had usually fallen to a shock level. The possible effect of anesthesia, hemorrhage, reflex inhibition of the vasoconstrictor center, exhaustion of the central nervous system with loss of vasomotor tone, acapnia and fat emboli were ruled out and the circulatory mechanism was then studied as the most reasonable point of attack of the liberated toxic material.

That the toxic material is carried in the circulation was demonstrated by Cannon in the following manner: the blood vessels of the leg (the iliac artery and vein) were ligated before the muscles were crushed, and the ligatures left in place for thirty-three minutes after the trauma, during which there was no fall in blood pressure. However, as soon as the blood flow was reestablished, the pressure promptly fell to a low level.

EXTRACT OF FRESH NORMAL LIVER

Extract of fresh normal liver was prepared by grinding the tissue with washed sand and extracting the mass with 0.9 per cent sodium chloride solution. The amount of saline added was equal to the original weight of the tissue used. After extracting for ten to fifteen minutes, the whole was filtered through cheesecloth. Fig. 1 is presented to show the effect of intravascular injection of such tissue extract, the record being obtained from a dog weighing 10.4 kilos and represents respiration and blood pressure. The animal received five injections of tissue extract, the dose increasing from 1 c.c. to 16 c.c. With each injection there is shown a definite blood pressure response. Yet promptly after each fall in blood pressure the pressure returned toward normal, there being apparently no prolonged toxic effect. This toxic action of normal tissue extract
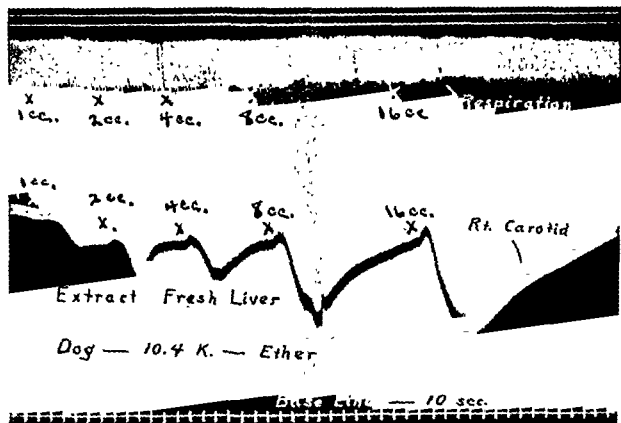
Fig. 1.

has been studied by various observers and has been ascribed to principally one of two causes or to a combination of the two: (1) the intravascular coagulation resulting from the intravenous administration of the tissue extract, and (2) the presence in the extracts of a substance which acts on smooth muscle. Wooldridge[3] made an extensive study covering the action of tissue extracts *in vivo*, and since his time numerous others have worked on the subject. (For literature see Smith[4] and Morse.[5]) The nature and action of the substance affecting the smooth muscle has been studied by Brieger and Uhlenhuth,[6] Dale,[7] Aronson,[8] Popielski,[9] Able and Kubota[10] and Smith[11]

EXTRACT OF AUTOLYZED LIVER

Fig. 2-*A* and *B* are the records obtained from two animals. The tracings show blood pressure and respiratory response to the injection of the extract of

autolyzed liver tissue. The extract was prepared in a manner similar to the extract of normal tissue, the autolyzed tissue being ground with sand, extracted with its weight of 0.9 per cent sodium chloride solution and filtered. In both tracings it will be noted, that the injection of the extract was accompanied by a marked response in both respiration and blood pressure. The amplitude of respiration becomes greater while the blood pressure shows a sudden fall followed by a rise. The rise in blood pressure is accompanied by a more rapid heart rate, shown in (A), and by an arrhythmia shown in (B). Such a response suggests the possibility that the toxic substance has a pro-
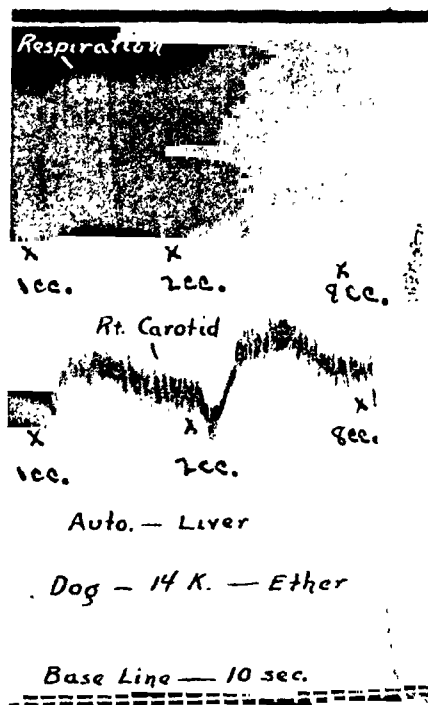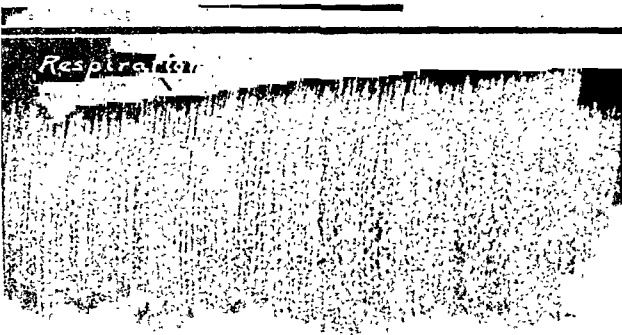
Fig. 2-A.   Fig. 2-B.

nounced action on circulation, the action being well shown in the latter part of (B) where 8 c.c. of the extract caused the sudden death of the animal.

Turck[12] has called attention to the fact that cell necrosis, brought about in various ways, produces results which are highly suggestive of shock symptoms. Delbet[13] has suggested that shock was due to the absorption of toxins arising from disorganized and bruised tissues. Delbet and Karajonopoulos[14] have reported the effects of injecting the product of muscle autolysis into guinea pigs. They found such autolysates very toxic causing an acceleration of respiration, loss of reaction to noise, and death of the animals in a variable time. The authors attributed these effects to disturbances of the nervous system.

Fig. 3.
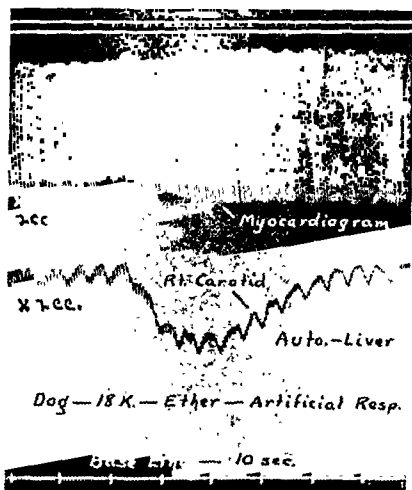
Fig. 4.

Fig. 3 is a record showing blood pressure record and myocardiogram obtained from a dog weighing 18 kilos, under ether anesthesia and maintained by artificial respiration. The method used for recording the myocardiogram is that described by Jackson.[15] It will be noted that during the fall in blood pressure the amplitude of the heart beat becomes less and apparently there is a slowing of the rate (better shown in Fig. 4.) However, during the rise in blood pressure, following the fall, the amplitude of the beat becomes increased and the rate more rapid.
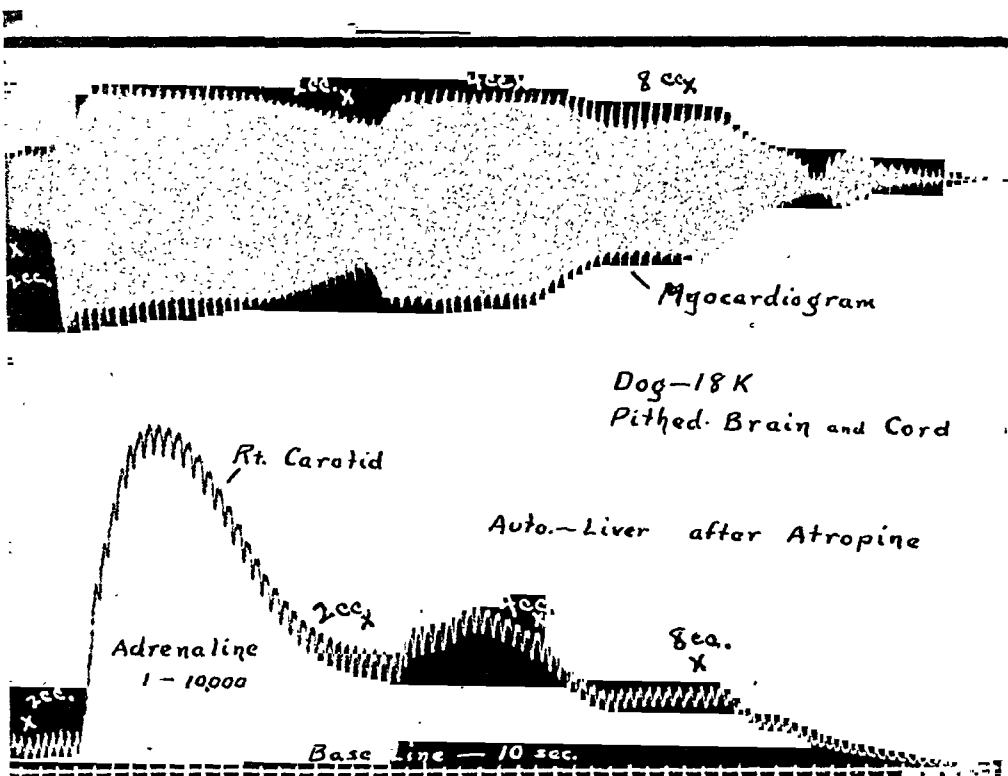
Fig. 5.

Fig. 4 was obtained from the same animal as Fig. 3 with all conditions the same except for an increase in the speed of the drum. It will be observed, that accompanying the fall in blood pressure, the heart beat became less rapid and showed less amplitude. (At the extreme left of the tracing there appears a slowing in the myocardiogram which is not really a slowing but is due to a change in the speed of the drum as shown by the individual heart beats in the blood pressure tracing.) Following the fall in blood pressure the myocardiogram shows an increase of volume and some increase of rate.

In attempting to obtain more definite information as to the point of action of the toxic substance we continued our studies with the use of atropine before the administration of the extract and have also destroyed brain and cord to rule out the possibility of central nervous system response. Fig. 5 is a record show-

ing some of the results obtained by such procedures. The record is one obtained from a dog weighing 18 kilos which had been pithed, brain and cord. He had also received four injections 1 c.c. each of atropine sulphate solution containing 1 mg. per c.c. The record represents blood pressure and myocardiogram. At the beginning of the tracing 2 c.c. of adrenalin (1-10,000) was administered. There is recorded the characteristic rise in blood pressure as well as an increase in the amplitude of the myocardiogram. As the adrenalin action wore off, 2 c.c. of extract of autolytic liver was injected, following which there was a slight
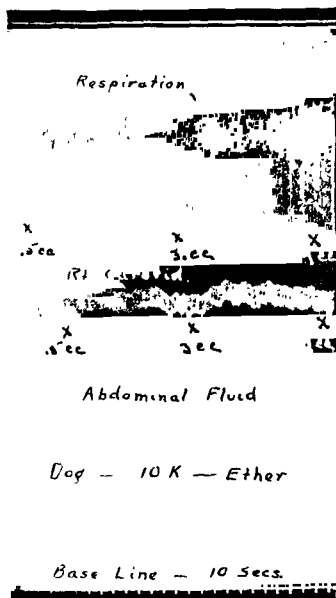
Fig. 6-A.
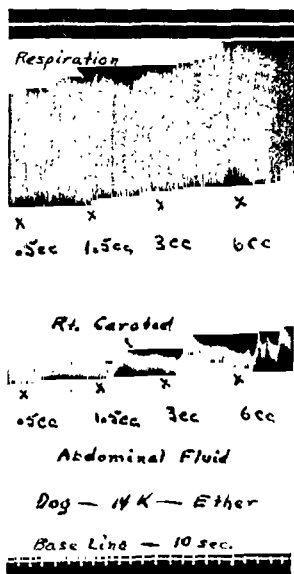
Fig. 6-B.

rise in blood pressure as well as an increase in amplitude of heart beat. After conditions became constant 4 c.c. of the extract was administered, and such an amount showed a decrease in myocardial action and a fall in blood pressure. Increasing the dose to 8 c.c. proved fatal to the animal. From this tracing it is evident that the toxic substance acts following the administration of atropine and pithing the brain and cord. The drop in blood pressure is not sudden as in the nontreated intact animal, and this suggests that the toxic substance may not act only on the myocardium but also, to a lesser degree, on the nerve structures. Cannon[2] in his study on shock has made observations with the cord transected and records his observations as follows: "It is only necessary to transect

the spinal cord above the lumbar plexus or to sever all nerves of the limb which is to be injured, in order to disconnect the region from the central nervous system. When this has been done and the denervated muscles are traumatized, events occur similar to those seen after trauma when the nerves are intact. From this evidence it is clear that there is no essential relation between the production of shock and an excessive stimulation of the central nervous system."

ABDOMINAL FLUID

Since the autolyzing liver proved so toxic, we assumed that the abdominal fluid present at the time of death should be quite toxic. We also thought that analysis of the abdominal fluid would probably suggest the nature of the toxic
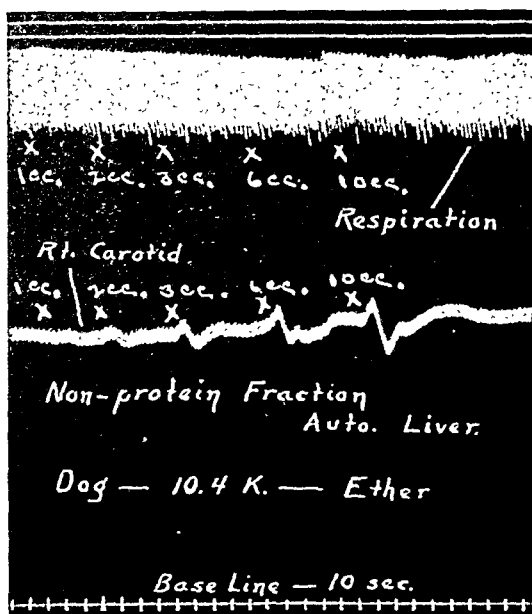
Fig. 7.

substance being absorbed. The following figures were obtained from the analysis of four abdominal fluids:

ANALYSIS OF ABDOMINAL FLUID

Nonprotein nitrogen	52.6	66.6		76.9
Urea nitrogen	33.5	42.0	29.4	35.2
Amino acid nitrogen	12.83	10.85	10.68	14.73
Sugar	35.	33.2	0.	181.8
Uric acid	2.48	2.66	2.4	3.15

It will be noted that all constituents, with the exception of sugar, were above the concentration found in the circulating blood. The variation in sugar is probably related to the time which autolysis has been under progress.

Figs. 6-A and B are presented to show the relative nontoxic action of the abdominal fluid administered intravenously. The principal action, especially in (B), appears to be in increasing blood pressure. The dose was increased to 12 c.c. in (B) but due to mechanical difficulties it does not appear on the record.

NONPROTEIN FRACTION

The salt extract of autolytic liver represents quite an indefinite mixture. Therefore we have made a very limited study with the proteins precipitated, using only the filtrate. Briefly: we used 30 c.c. salt extract of the autolyzed liver, added 30 c.c. 5 per cent sodium tungstate, 30 c.c. three-fourths normal sulphuric acid and filtered. Blood was obtained from the animal to be used and 7 c.c. was added to the tungstate filtrate, the whole well shaken and filtered. The filtrate was neutralized to phenolphthalein by adding 15 c.c. of tenth normal sodium hydroxide. Fig. 7 is a record of the blood pressure and respiratory response to the injection of such filtrate. While it is true the response is relatively slight, it should be remembered that the tissue extract has been diluted to approximately four times its original volume during the preparation.

CONCLUSIONS

1. Intravenous administration of the saline extract of autolyzed liver demonstrates the material to be highly toxic.

2. Autolyzed liver tissue contains a toxic substance which apparently is not present in normal liver extract.

3. The toxic substance apparently kills by its action on the circulatory mechanism, such action being principally on the myocardium with possibly a slight action on the nerve structures.

4. Analysis of the abdominal fluid, present at the death accompanying autolysis, shows the fluid to be higher in nonprotein nitrogen, urea, uric acid, and amino acid than the circulating blood, but usually lower in sugar.

5. The abdominal fluid is relatively much less toxic than the autolyzed liver.

6. After a limited study it appears that the toxic material is partially if not wholly present in the nonprotein nitrogen fraction.

7. There appears to be a relation between our observations and those reported by Cannon in his studies on "Wound Shock."

REFERENCES

[1]Mason and Davidson: JOUR. LAB. AND CLIN. MED. (In press.)
[2]Cannon: Arch. Surg., 1922, iv, 1.
[3]Wooldridge on the Chemistry of the Blood and other Scientific Papers, London, 1893.
[4]Smith: JOUR. LAB. AND CLIN. MED., 1919, iv, 517.
[5]Morse: JOUR. LAB. AND CLIN. MED., 1919, v, 319.
[6]Brieger and Uhlenhuth: Deutsch med. Wchnschr., 1893, xxiv, 163.
[7]Dale and Coworkers: Jour. Physiol., 1910, xl, 503; ibid., 1910, xli, 318; ibid., 1911, xli, 499; ibid., 1918, lii, 110; ibid., 1919, lii, 355.
[8]Aronson: Berl. klin. Wchnschr., 1913, 1, 253.
[9]Popielski: Bull. internat. Acad. d. sc. de Cracovie, 1912, p. 1157 (Chem. Abstr., viii, 1463).
[10]Able and Kubota: Jour. Pharmacol. and Exper. Therap., 1919, xiii, 283.
[11]Smith:[4]
[12]Turck: Med. Rec., 1919, xcv, 472; ibid., 1918, xciii, 927.
[13]Delbet: Bull. et mém. Soc. de chir. de Par., 1917, xliii, 2121.
[14]Delbet and Karajonopoulos: Bull. Acad. de méd., 1918, lxxx, 14.
[15]Jackson: Exper. Pharmacol., St. Louis, 1917, C. V. Mosby Co., p. 149.

STUDIES WITH B. ACIDOPHILUS AND B. BULGARICUS

I. PRELIMINARY ATTEMPTS AT IMPLANTATION OF B. BULGARICUS*

BY NICHOLAS KOPELOFF, PH.D., AND PHILIP BEERMAN, WARD'S ISLAND, N. Y.

THE failure of B. bulgaricus in the United States and the subsequent success of B. acidophilus therapy, raises many interesting clinical and bacteriologic problems. Herter and Kendall,[1] in 1908, were unable to establish the predominance of B. bulgaricus in the ileocecal region or large intestine of monkeys after exclusive feeding of bacillac milk. Rahe[2] demonstrated that while an apparently limited survival of B. bulgaricus could be obtained in the upper intestine of monkeys, nevertheless, this organism could not become adapted to the human lower intestine, despite its occasional recovery after its administration has been stopped. The comprehensive experiments of Rettger and Cheplin[3] finally sealed the fate of B. bulgaricus and stimulated further interest in B. acidophilus.

It should be noted, however, that conclusive as the aforementioned data appear, Rahe's work was based on four human subjects and Rettger and Cheplin's on four human subjects. In view of the favorable implantation of B. bulgaricus reported by Cohendy[4] and Belonovsky,[5] as well as the continued use of Metchnikoff's B. bulgaricus therapy in France, there would still seem to be a place for further studies on a larger scale. This is particularly true since the differentiation of B. bulgaricus from B. acidophilus remains a vexing problem.

In morphology and cultural characteristics these closely related organisms are similar. Heinemann and Hefferan[6] claimed that B. bulgaricus was widely distributed in nature; in feces from cows, horses, and man, in human fluids, etc., and that "some bacilli isolated from various fermented milks, so-called acidophile bacteria found in the intestinal tract of man, Boas-Oppler bacillus, B. panis fermentati, St. Lebenis, Leptothrix bucalis and possibly B. bifidus of Tissier are identical with B. bulgaricus." On the other hand, Rahe[7] established that B. acidophilus ferments maltose, while B. bulgaricus does not. This important distinction has been accepted by most of the investigators in this field; Rettger and Cheplin,[3] Cannon[8] and others. Kulp and Rettger[9] in a comparative study have noted many variations in fermentative power, although they state that "the action upon maltose, sucrose and levulose appears to furnish a valuable means of separating strains of these two groups. In addition to being unlike in their fermentative action upon the three sugars mentioned, they differ in another very important respect, namely, in that L. acidophilus can and does develop in the intestine of man and animals

whereas, L. bulgaricus is unable to do so.'' Recently, Cruickshank and Berry[10] have shown that of 52 strains of B. acidophilus, isolated from human feces, 44 did not ferment maltose and 4 did not ferment glucose.

The present study grew out of an investigation which one of us carried on at the Pasteur Institute, in Paris, in the Department of Physiological Chemistry under G. Bertrand and this opportunity is taken to express my deep appreciation of the courtesies extended. While data there obtained, using rats, have not yet been published, in the main they corroborated the findings of Rahe[2] to which reference has been previously made. However, they were equivocal enough to require further investigation, particularly as regards the implantation of B. bulgaricus in the human intestine. Therefore, a group of 12 women (psychotic) were selected for fecal examination and administration of B. bulgaricus milk.

The procedure was as follows: Two or more fecal examinations were made on about 35 women (psychotic) to select a group in which no colonies showing the typical morphology of B. acidophilus or B. bulgaricus were found on whey agar plates. Seven constipated and five nonconstipated subjects were thus selected for administration of milk fermented by B. bulgaricus. The cultures used for this purpose were: A strain called the Pasteur Institute bacillus corresponding to Metchnikoff's original culture, kindly furnished by Legros of the Pasteur Institute, Paris; and two strains used by Kulp and Rettger, labelled B-3 and B-4 respectively, which Kulp of Yale University was kind enough to furnish. The technic of preparing milk, plating, etc., was the same as described by us in employing B. acidophilus milk, except that the counts averaged about 50,000,000 viable organisms per cubic centimeter.[11]

The twelve subjects were arranged in groups of four; two constipated, and two nonconstipated, to receive milk fermented by the same strain of B. bulgaricus (an exception to this plan was the substitution of a constipated subject, M. Fy. for a nonconstipated one). The administration of one liter of bulgaricus milk per day in addition to the regular hospital diet was carried on with the Pasteur Institute bacillus for fifteen days, with B-3 for twelve days and with B-4 for ten days. Three fecal examinations at three-day intervals were made during treatment and two after treatment. Counts were made from the dilutions, 1:100,000 and 1:1,000,000.

During the treatment, there were only two subjects showing B. bulgaricus. One subject, T. Co., had 20 per cent after one week of treatment and 10 per cent after thirteen days of treatment. Subsequently no B. bulgaricus were found in her feces. The other subject, D. Pr., showed 50 per cent of B. bulgaricus, after one week of treatment and 60 per cent after ten days. The day, after treatment had been discontinued, this was the only subject to have B. bulgaricus present. There were 80 per cent found. However, they disappeared in the following seven days, when no B. bulgaricus was recovered. It is significant that both these subjects were nonconstipated to begin with. No change was noted in the color, character, or consistency of the feces from these subjects although daily observations were recorded.

One might be led to conclude from these data that they corroborated previous investigations which established that B. bulgaricus is not implanted

in the human intestine. However, such a conclusion might be premature without first considering the dosage. It has been stated that the fermented milk averaged about 50,000,000 viable organisms per cubic centimeter. Elsewhere[12] the importance of the number of viable organisms per cubic centimeter of milk has been stressed. Suffice it to say that any conclusion to be derived from the foregoing data should be considered in the light of the number of viable organisms present. It is conceivable that an implantation of B. bulgaricus, might occur with larger amounts such as 200,000,000 per cubic centimeter, as previously employed by us in B. acidophilus milk. The plan of this experiment, of which this represents but the first step, calls for a repetition of the administration of milk fermented by B. bulgaricus which will contain larger numbers of viable organisms. Finally, after discontinuing treatment with B. bulgaricus, those subjects who have not had any B. bulgaricus in their feces will be given B. acidophilus milk. In this way further evidence may be added to the crucial question of whether B. bulgaricus can be implanted in the human intestine.

It is a privilege to acknowledge our indebtedness to George H. Kirby, Director of the Psychiatric Institute and to Helen Langner of the Manhattan State Hospital.

SUMMARY

Subject to the limitations of the data, it has been shown in this preliminary study, that:

1. Of 12 human subjects receiving 1 liter of B. bulgaricus milk for from ten to fifteen days, only 2 revealed the presence of B. bulgaricus in the feces. In one of these subjects the number of B. bulgaricus present was so small as to be negligible.

2. Since the bacterial content of the milk fed was only 50,000,000 per cubic centimeter, it is believed that further corroboration of the failure to implant B. bulgaricus is required, when using the same dosage of milk containing about 200,000,000 per cubic centimeter, as is recommended in the feeding of B. acidophilus.

REFERENCES

[1]Herter, C. A., and Kendall, A. I.: An Observation on the Fate of B. Bulgaricus (in Bacillac) in the Digestive Tract of a Monkey, Jour. Biol. Chem., 1908, v, 293-302.
[2]Rahe, A. H.: A Study of the So-Called Implantation of the Bacillus Bulgaricus, Jour. Infect. Dis., 1915, xvi, 210-220.
[3]Rettger, L. F., and Cheplin, H. A.: A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of Bacillus Acidophilus, Yale University Press, 1921.
[4]Cohendy, M.: Description d'un ferment lactique puissant capable de s'acclimater dans l'intestin de l'homme, Compt. rend. Soc. de biol., 1906, lx, 558-560.
[5]Belonovsky, G. D.: Sur la prolongation de la vitalite' du Bacille Bulgare, Compt. rend. Soc. de biol., 1913, lxxv, 374-375.
[6]Heinemann, P. G., and Hefferan, M.: A Study of B. Bulgaricus, Jour. Infect. Dis., 1909, vi, 304-318.
[7]Rahe, A. H.: An Investigation into the Fermentative Activities of the Aciduric Bacteria, Jour. Infect. Dis., 1914, xv, 141-150.
[8]Cannon, P. R.: A Biologic Study of Aciduric Bacteria, Jour. Infect. Dis., 1924, xxxiv, 227-238.
[9]Kulp, W. L., and Rettger, L. F.: Comparative Study of Lactobacillus Acidophilus and Lactobacillus Bulgaricus, Jour. Bacteriol. 1924, ix, 357-394.

[10]Cruickshank, J., and Berry, D. W.: A Study of B. Acidophilus in Human Feces, Brit. Med. Jour., Nov. 22, 1924, No. 3334, 914-948.
[11]Kopeloff, N., and Cheney, C. O.: Studies on the Therapeutic Effect of Bacillus Acidophilus Milk and Lactose, Jour. Am. Med. Assn., 1922, lxxix, 609-611.
[12]Kopeloff, N.: Clinical Results Obtained with Bacillus Acidophilus, Arch. Int. Med., 1924, xxxiii, 47-54.

PHYSICOCHEMICAL ASPECTS OF HEMOLYSIS

III. A FURTHER STUDY OF SOME OF THE FACTORS INVOLVED*

BY REED ROCKWOOD, M.D., AND EDWARD C. MASON, M.D., ROCHESTER, MINNESOTA

IN a previous paper on this subject, we[5] have called attention to the fact that the various reactions of the red cell favor the conclusion that it is essentially a mass of colloidal components, the chief colloids from the standpoint of hemolysis being the stroma and the hemoglobin. We further expressed the belief that the stroma-hemoglobin union (red cell) was of a physical nature, and possessed many of the characteristics of an adsorption phenomenon. Our studies with such combination led us to summarize our interpretation as follows: "All agents which tend to change the red cell from a state of (1) small mass, (2) low hydration capacity, (3) maximal cohesion, and (4) relative electrical neutrality to a state of (1) large mass, (2) increased hydration capacity, (3) decreased cohesion (which is accompanied by decreased surface tension), and (4) away from the isoelectric point, do to the same degree promote hemolysis."

In the present study we have extended our observations to include cell volume changes in the presence of various hemolytic agents, and viscosity changes during biologic hemolysis; that is, during the reaction of cells, amboceptor, and complement.

THE RELATION OF CELL VOLUME TO HEMOLYSIS

The literature on this subject exhibits somewhat conflicting data. Fischer, on theoretic grounds, believes that volume changes and loss of hemoglobin are separate processes which only happen to parallel each other at times. He believes that hemolysis by hypotonic solutions, acids and alkalis is probably accompanied by some swelling of the cell colloids, but not of other forms. Ponder, by the use of a formula which determines the approximate cell volume from measurements of the diameter, concludes that the volume of cells hemolyzed by hypotonic solutions was about 448 cubic micra at the time of hemolysis, while with saponin it was 131 cubic micra, and with sodium taurocholate 187 cubic micra, the normal cell having a volume of about 70 cubic micra. In biologic hemolysis (cells, amboceptor, and complement) he noted no change. Von Knafll-Lenz made hematocrit readings on cells and found no change in cell

volume on the addition of saponin. The cell volume decreased or was not altered with the use of ethyl alcohol, ether, urethane, salicylamide, benzamide, camphor or urea in small quantities, on account of the loss of water, and thus these substances opposed hemolysis by hypotonic solutions. In higher concentrations they caused swelling. Brahmachari, in hematocrit studies, found no change in cell volume on the addition of amboceptor. We have performed the following experiments with the hope of adding to our knowledge of the relation between cell volume and hemolysis.

METHOD OF STUDY

Fresh washed beef cells were placed in graduated centrifuge tubes with different amounts of the hemolytic agent, and the volume made up to 10 c.c.
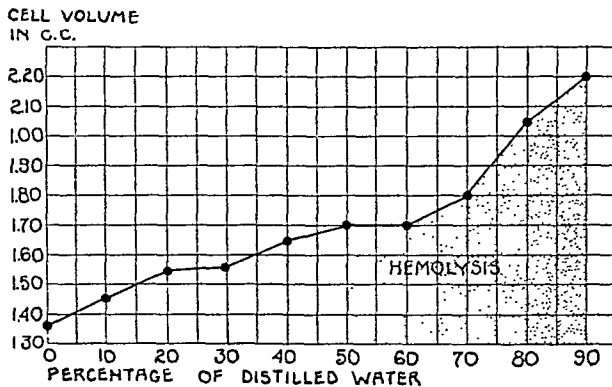
Fig. 1.—Hemolysis by distilled water.
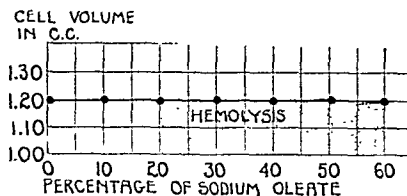
Fig. 2.—Hemolysis by sodium oleate. 1:1000.

with isotonic salt solution. They were then centrifugalized at constant speed at room temperature (25° to 30°) for ten minutes at about 2000 revolutions, the tubes in each group of tests being in the centrifuge at the same time. The volume of the cells in the bottom of each tube was then read. The concentrations of the hemolytic agents used were designed to cause, at the most, a slight hemolysis. A greater degree of hemolysis would destroy too many cells, thus decreasing the cell volume so that no results of value could be obtained. Of course it is obvious that this method measures the alterations in the cell mass only, not the change in the individual cell which occurs as it hemolyzes.

RESULTS

The results of this method are exemplified in Fig. 1, which shows the marked increase in cell volume during hemolysis by distilled water in varying

proportions in isotonic salt solution. On the other hand, amboceptor and complement, 1:1000 sodium oleate, and 1:1000 saponin in isotonic salt solution showed no significant variation. Fig. 2 is representative of this group.

Hemolysis by autolysis, that is, by standing for several hours, can be prevented by the addition of hypertonic sodium chloride. This has been discussed by one of us in a previous paper. Figs. 3 and 4 show the changes in cell volume which accompany this phenomenon. In the protected zone the hypertonic salt solution causes a decrease in volume, while the autolyzing cells and those hem-
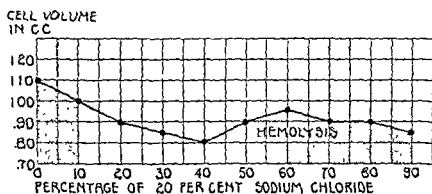
Fig. 3.—Inhibition of autolytic hemolysis by hypertonic salt solution   Time, sixteen hours.
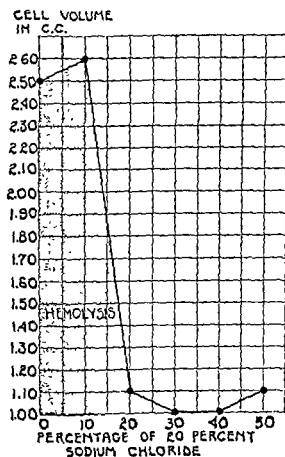
Fig. 4.—Inhibition of autolytic hemolysis by hypertonic salt solution.   Time, eighteen hours.

olyzed by the highest concentrations of sodium chloride show an increase in volume.

Variable results are secured with the use of hydrochloric acid. In some cases there is a swelling, the most marked increase in total volume of unhemolyzed cells and stroma being 44 per cent. More frequently only a slight swelling was observed; sometimes none at all, hemolysis still occurring. This would tend to confirm Fischer's general concept that cell swelling and hemolysis are

independent processes. In using sodium hydroxide in sufficient concentration to cause beginning hemolysis, no significant alteration in cell volume was noted.

The P_H of the solutions was determined colorimetrically, after pipetting off the supernatant fluid, by matching them with the Clark and Lubs standards. Owing to hemolysis, the reading in some tubes was difficult, and can only be considered as approximate. Because of the lack of buffer, the solution could not be diluted to decrease the color of the hemoglobin. In the experiments with phosphate buffer, dilution could be made, and hence the readings on buffered solutions with hemolysis are much more accurate. Tables I and II are typical of the results obtained with acid and alkali.

An experiment with isotonic phosphate buffers ($M/7$ $KH_2 PO_4$ and $M/8.8$ $Na_2 HPO_4$, $2H_2 O$) is shown in Table III. Such experiments show no marked variation in cell volume. We are unable to interpret the slight tendency for the
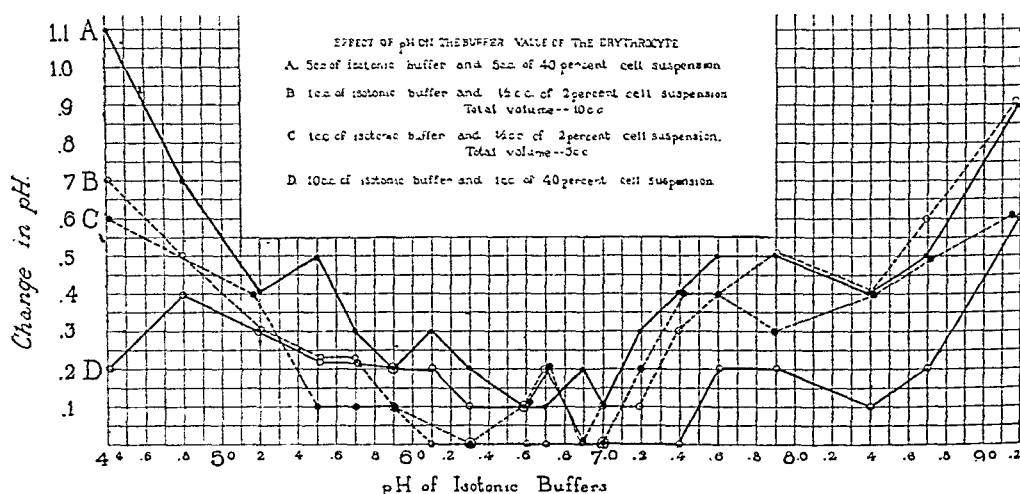
Fig. 5.—Effect of P_H on the buffer value of the erythrocyte.

cell volume to decrease toward the alkaline end of the range, but we have seen it in other similar experiments. It will be noted that hemolysis increases with acidity, alkalinity, and dilution of the salt.

THE BUFFER VALUE OF THE ERYTHROCYTE AND ITS RELATION TO HEMOLYSIS

It may be noted in Fig. 5 that the addition of cells has considerable buffering effect on the phosphate solutions; this is not uniform at each P_H; the amount of buffering depending on the P_H of each solution, the most marked effect being at the extreme acid and alkaline sides of the range. This observation has been confirmed in a number of instances. Thus, the experiment in our original paper on the effect of acidity on hemolysis has been repeated, and the actual P_H in each tube has been determined colorimetrically after centrifugalizing. Isotonic phosphate buffers were used in this case, and the general results as far as hemolysis is concerned were the same as those previously reported. Small amounts of cells can apparently also exercise a considerable buffer power. Thus 1 c.c. of isotonic buffer and 0.5 c.c. of 2 per cent sheep cells in a total of 3 c.c. of fluid (0.3 per cent suspension) show a marked buffer action in extremely acid and

TABLE I

EFFECT OF ONE-FIFTIETH NORMAL HYDROCHLORIC ACID ON CELL VOLUME

TUBES	1	2	3	4	5	6	7	8
Isotonic sodium chloride, c.c.	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5
Acid	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Hemolysis	0	0	*	**	**	***	***	+
Volume	2.6	2.6	2.5	2.6	2.6	2.6	2.6	2.7
P_H	7.2	7.4	6.8	6.7	6.6	6.4	6.0	6.0

*Very faint trace. **Faint trace. ***Trace. +Considerable hemolysis.

TABLE II

EFFECT OF ONE-TWENTIETH NORMAL SODIUM HYDROXIDE ON CELL VOLUME

TUBES	1	2	3	4	5	6	7	8
Isotonic sodium chloride, c.c.	5.0	3.0	2.5	2.0	1.5	1.0	0.5	0
Alkali	0	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Hemolysis	0	0	0	0	*	*	**	+
Volume	2.3	2.2	2.2	2.2	2.1	2.2	2.3	2.3
P_H	7.2	10.0 to 10.5	12.1 to 12.7	12.1 to 12.7	12.1 to 12.7	12.1 to 12.7	Greater than 12.7	

*Faint trace. **Trace. +Considerable hemolysis.

alkaline ranges. This is the amount of cells and volume of fluid used in the Kolmer technic for the Wassermann reaction. These experiments were repeated, making the total volume 10 c.c.; the resulting curve was the same. These four curves of buffer value are shown in Fig. 5.

It is obvious from these curves that the maximal buffering power of the cell is not exerted, except at the extremes of acidity and alkalinity. This means that in the buffering which occurs at ordinary ranges all the cell buffers are not

TABLE III

EFFECT OF ISOTONIC PHOSPHATE BUFFERS ON CELL VOLUME AND HEMOLYSIS

Each Tube contains 5 c.c. of buffer and 5 c.c. of cell suspension

P_H OF BUFFER	4.4	4.8	5.2	5.5	5.7	5.9	6.1	6.3	6.6	6.9	7.2	7.4	7.6	7.9	8.4	9.2
Hemolysis	*	**	***	0	0	0	0	0	0	0	0	0	0	0	0	0
Cell volume	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.0	2.0	1.9	1.9	1.8	1.8	1.8	1.8
Correct P_H	5.5	5.5	5.6	6.0	6.0	6.1	6.4	6.5	6.5	6.7	6.9	7.0	7.1	7.4	8.0	8.3

1 C.C. OF DISTILLED WATER ADDED

Hemolysis	+	+	+	†	†	*	*	**	***	0	0	0	0	0	0	0
Cell volume	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.0	2.0	2.0	1.9	1.8	1.8	1.8	1.9	1.8

1 C.C. OF DISTILLED WATER ADDED

Hemolysis	2+	2+	2+	+	+	†	†	†	*	**	0	0	0	0	0	**
Cell volume	1.8	1.8	1.9	2.0	1.9	2.1	2.1	2.1	2.1	2.1	2.0	1.9	1.9	1.9	1.9	1.9

1 C.C. OF DISTILLED WATER ADDED

Hemolysis	3+	3+	3+	2+	2+	+	+	+	†	*	**	**	0	0	0	**
Cell volume	1.4	1.4	1.4	1.6	1.6	1.8	1.8	1.9	2.0	2.0	2.0	1.9	1.9	1.9	1.9	1.9

1 C.C. OF DISTILLED WATER ADDED

Hemolysis	4+	4+	4+	3+	3+	2+	2+	2+	+	†	†	†	*	**	**	*
Cell volume	0.7	0.7	0.7	1.0	1.0	1.3	1.4	1.4	1.8	2.0	2.1	2.0	2.0	2.0	2.0	1.9

*Faint trace. **Very faint trace. ***Slightest possible trace. †Trace.
+Considerable hemolysis.

available, while another source can be made effective as the acidity or alkalinity increases. The most important cell buffers are the bicarbonate and cell proteins. The cell phosphate is apparently of little importance. It would seem most likely that the proteins of the cell are responsible for the peculiarities of the buffer curves, and form the final reserve. The relative importance of hemoglobin and stroma protein cannot be determined. However, Van Slyke, Hastings, and Neill have calculated that for oxygenated blood the hemoglobin was responsible on an average, for 76.0 per cent of the total buffer value, and the bicarbonate for 6.9 per cent of it. In reduced hemoglobin the figures were 73.3 per cent and 9.0 per cent respectively. This work involves the assumption that the confinement of hemoglobin within the red cells does not alter its buffer value in the system. The relative preponderance in mass of hemoglobin over protein in the stroma suggests also its predominant importance.

The similarity of these curves of buffer action to the curves showing the action of acid and alkali in causing hemolysis suggests the possibility of a causal connection between the two. It might be postulated that the change which takes place in the hemoglobin, and possibly also in the stroma protein in an attempt at buffering the solution so alters these proteins that the hemoglobin stroma union is rendered unstable. It should be recalled in this connection that it would be possible by increasing the acidity of the solution to carry these proteins past their isoelectric points, and thus reverse their charges. This could not be done by putting them in more alkaline solutions, since they are normally on the alkaline side of their isoelectric points. But again it has been shown that the cell is much more easily hemolyzed by acids than by alkalis.

VISCOSITY AND BIOLOGIC HEMOLYSIS

Assuming that the red cells, amboceptor, and complement unite during the course of the biologic hemolysis, it seemed reasonable to us that the resulting state should represent fewer particles, and consequently should have less viscosity. On the other hand, an increase in the hydration capacity of such a system would lead to an increase in viscosity. Therefore, it is evident that the resulting viscosity would probably depend on the balance of these two phenomena.

Viscosity determinations were made on each of the components of the biologic hemolytic system, separately, and combined. The apparatus used was that designed by Hess, which has certain advantages, owing to the horizontal position of the capillaries which eliminates the influence of specific gravity, and, since the water and the colloid are tested simultaneously, there is no need for temperature corrections. The differences between combinations of the three reagents and complement alone were so slight, however, as to make it impossible to draw any definite conclusions.

THE HEMOLYSIS OF AGAR HEMOGLOBIN COMBINATIONS

In our previous paper, we discussed the relationship of hemoglobin and kaolin combinations to their union and dissociation. We have also obtained similar results by substituting an emulsoid, such as agar, for a suspensoid like kaolin.

An agar jelly was made of 12.5 gm. of agar to 1000 c.c. of 0.85 per cent

sodium chloride. The agar was cooled to just above the point of solidification, and to each 100 c.c. was added 20 c.c. of phosphate buffer of known P_H, and 10 c.c. of hemoglobin solution made by hemolyzing washed red blood corpuscles in distilled water. After the agar-hemoglobin mixtures had cooled, they were ground in a mortar at room temperature as finely as possible, and allowed to stand overnight. They were then washed in isotonic sodium chloride solution

TABLE IV

"HEMOLYSIS" OF HEMOGLOBIN-AGAR COMPLEX

P_H OF SOLUTION IN TUBE	P_H OF AGAR PARTICLES					
	6.2	6.6	6.9	7.3	8.0	9.2
4.4	+	-	-	+ +	+ + + +	+ + + +
5.5	+	+	+ +	+ + +	+ + +	+ + + +
6.2	+ +	+	+ +	+ +	+ + +	+ + + +
6.6	+ + +	+	+	+ +	+ + +	+ + +
6.9	+ +	+	+	+	+ +	+ +
7.3	+	+ + +	+ + +	+	+	+ +
8.0	0	+ + +	+ +	0	-	+
9.2	+ + +	+ +	+ +	+ +	+	+

+Indicates degree of hemolysis.

DISCHARGE OF PIGMENT FROM THE BEET CELL
●——● BUFFER SOLUTION
●--● BUFFER SOLUTION AND 10 C.C OF DISTILLED WATER
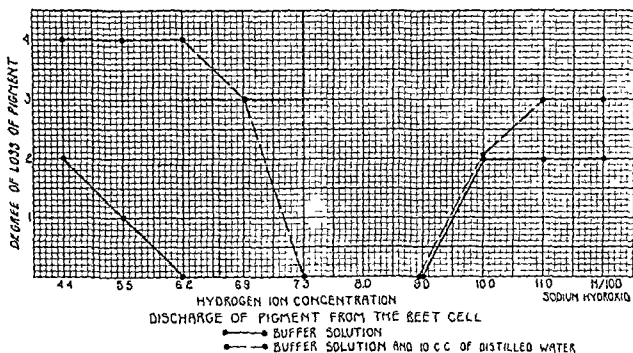
Fig. 6.—Hydrogen-ion concentration   Discharge of pigment from the beet cell.

until the solution was nearly clear. Two grams of agar particles were then added to 20 c.c. of buffer phosphate solution in a test tube, and the process of hemolysis observed. The amount of hemoglobin released was graded approximately by matching the tubes from 1 to 4. The two buffers of highest acidity, that is, P_H 4.4, and 5.5, were acid enough to change the hemoglobin to dark brown. There was no discharge of pigment, and hence these buffers could not be used.

It will be seen from the results in Table IV that there is a distinct tendency for the hemoglobin to separate from the acid mixtures when they are placed in alkaline solutions, and from the extremely alkaline mixtures when placed in acid solutions. The mixtures which were originally near the neutral point, like the hemoglobin-kaolin mixtures previously described, and the red blood corpuscles themselves "hemolyze" in the presence of both acid and alkali. Fischer's

experiments with combinations of fibrin and carmin are of great interest in this connection.

These findings are somewhat analogous to those of Kosaka and Seki with the cells themselves. They believe that the corpuscles which were most strongly charged negatively were most easily dissolved in acid, and that the least powerfully charged dissolved most readily in alkali.

The results in our experiments apply to other cells as well as to the red blood corpuscles. Thus, Fig. 6 shows the relation of acidity and salt concentration to the discharge of the pigment from the cell of the red beet. The curves are strikingly similar to those observed in the case of hemolysis and the dissociation of hemoglobin-colloid complexes. They were secured by placing cubes of beet in buffer phosphate solution and diluting the mixtures up to varying degrees. The exact location of the pigment in the beet cell has not been carefully worked out. The best information appears to be that some of it is present in the vacuoles, while another portion may be bound to other constituents of the protoplasm, or even present in crystalline form.

CONCLUSIONS

1. Under the conditions of the experiment described, hemolysis by hypotonic solutions is accompanied by swelling of the cell mass, while hemolysis by alkali, complement and amboceptor, saponin and sodium oleate shows no change in the cell volume. Hemolysis by acid may or may not be accompanied by swelling.

2. The cells have considerable buffer power; it is not the same at every P_H, but is greatest at the extremes of acidity and alkalinity. This suggests a combination of acid and alkali with an additional buffer substance in these parts of the range, and it is suggested that this combination may play an important part in rendering the hemoglobin-stroma union unstable in these zones.

3. Viscosity determinations on the components of the biologic hemolytic system show no significant change during hemolysis.

4. The hemoglobin and agar mixtures show a certain similarity to the corpuscles in their behavior toward acid and alkali.

5. The discharge of pigment from the cell of the red beet under the influence of acid, alkali, and salt is in many respects similar to loss of hemoglobin from the red cell or from the hemoglobin-colloid complexus.

REFERENCES

[1] Brahmachari, U. N.: An Investigation into the Physico-chemical Mechanism of Hemolysis by Specific Haemolysins, Biochem. Jour., 1913, vii, 562-567.

[2] Fischer, M. H.: Edema and Nephritis. A Critical Experimental and Clinical Study of the Physiology and Pathology of Water Absorption in the Living Organism, New York, 1915, Wiley and Sons, pp. 695.

[3] Knaffl-Lenz, v. E.: Über die colloidchemischen Vorgänge bei der Hämolyse, Arch. f. d. ges. Physiol., 1918, clxxi, 51-65.

[4] Kosaka, K., and Seki, M.: Electric Charges of the Red Blood Corpuscles, Chem. Abstr., 1923, xvii, 1825.

[5] Mason, E. C., and Rockwood, Reed: Some Physicochemical Aspects of Hemolysis, JOUR. LAB. AND CLIN. MED., 1924, x, 10-18.

[6] Ponder, E.: Alterations in the Diameters of the Erythrocytes During Hemolysis, Proc. Roy. Soc. Med., London, 1922, s. B., 102-108.

[7] Rockwood, Reed: Physicochemical Aspects of Hemolysis. II. An Ultramiscroscopic Study of Hemolysis, JOUR. LAB. AND CLIN. MED., 1924, x, 19-31.

[8] Slyke, van D. D., Hastings, A. B., and Neill, J. M.: Studies of Gas and Electrolyte Equilibria in the Blood. IV. The Effect of Oxygenation and Reduction on the Bicarbonate Content and Buffer Value of Blood, Jour. Biol. Chem., 1922, liv, 507-526.

LABORATORY METHODS

A METHOD FOR INSTANTANEOUS PHOTOMICROGRAPHY OF THE SKIN CAPILLARIES*

By Charles Sheard, Ph.D., and George E. Brown, M.D.,
Rochester, Minnesota

THE clinical investigation of the capillaries of the skin has opened up a new field of vascular research which may be of value both from physiologic and clinical standpoints. In health and in certain diseases these studies have enlarged our knowledge of the disturbances which take place in this important part of the vascular field. Capillary studies have been directed toward defining form, size, tonus, flow, and reaction to stimuli. While many of these studies relative to size have been of a quantitative nature, employing the eyepiece micrometer, still considerable error has existed, and permanent and accurate records have been lacking. This has been true particularly in obtaining accurate data relative to the capillary caliber. The quantitative determinations of the caliber in these vessels may be useful as an index of capillary tonus and permeability, for Krogh has shown that permeability and dilatation of the capillaries are directly related. The capillary response to various agents, as well as data on measurements of the visible capillary loop or loops, can be more accurately determined from photographic records. The number of functioning capillaries for each unit area can also be made a matter of record, thus securing a quantitative index of the metabolic activity of the skin. The determination of the capillary flow is likewise of importance. It is possible that a method may be worked out for determining this more accurately than the present methods permit, whereby the passage of the leucocyte can be imaged, timed, and measured by means of multiple photographs.

DIFFICULTIES IN THE PHOTOMICROGRAPHY OF THE SKIN VESSELS

Several workers have attempted to utilize a practical method of photographing the vessels of the skin, but there have been certain fundamental errors and difficulties involved which have prevented their further application. Little light is needed to make the capillaries microscopically visible to the observing eye. The problem from the photographic standpoint, however, is much more difficult, owing chiefly to (1) inability to get sufficient light reflected from the capillaries and returned to the photographic plate or film so that photomicrographs can be obtained in a fraction of a second; (2) with longer exposures, of the order of a few seconds, the difficulty of preventing mechanical movements of the finger or apparatus, and eliminating the action

of heat and actinic rays on the vessels; (3) the impossibility of preventing slight rhythmic movements due to arterial pulsations and (4) the difficulty of coordinating the eye and plate focus.

Preliminary experiments* demonstrated the inadvisability of endeavoring to overcome difficulties (2) and (3), and indicated that success in instantaneous photomicrography of the capillaries would result only through the use of intense external illumination, so filtered as to remove heat radiation, and directed at such an angle as to throw the light reflected by the surface of the skin out of the path of the light reflected by the capillaries and, in turn, trans-
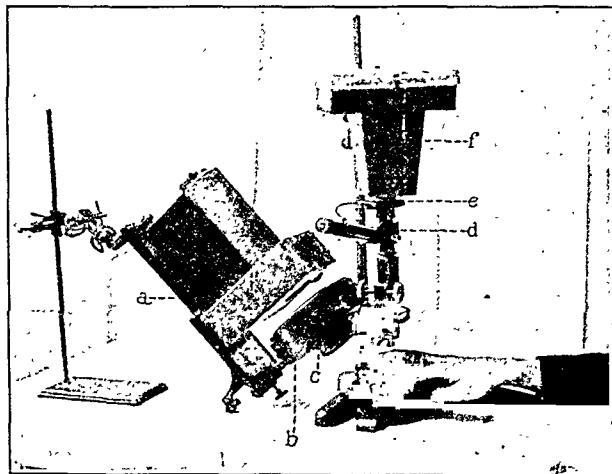
Fig. 1.—Showing ensemble of photographic apparatus. Arc lamp, *a*; water-copper chloride filter, *b*; lens system, *c*; demonstration eyepiece, *d*; shutter, *e*; camera, *f*.
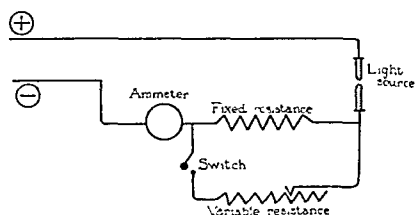
Fig. 2.—Diagrammatic sketch of electrical circuit. Ammeter; fixed resistance; variable resistance to throw in parallel with fixed resistance; switch; and carbon arc.

mitted to the photographic plate or film. Other difficulties also arise by reason of the character of the oil used, the texture of the skin, the presence of moisture under the oil film caused by sweating, the condition of the cuticle and the slight readjustments of focus necessary under the higher illumination used when photographing.

METHOD

The essential pieces of apparatus are shown in Fig. 1. All electrical connections and other similar details, familiar to electricians, are omitted. Fig. 2 gives a diagrammatic sketch showing the details of the electrical circuit.

*Sheard, Charles: Instantaneous Photomicrography of the Skin Capillaries in the Living Human Body, Science, 1924, lx, 409-410.

The light source is a direct current arc (Fig. 1, a) running at 5 amperes or thereabouts during the period of visual observation, with suitable resistances (for example, a variable resistance obtained by a carbon rheostat) ar-
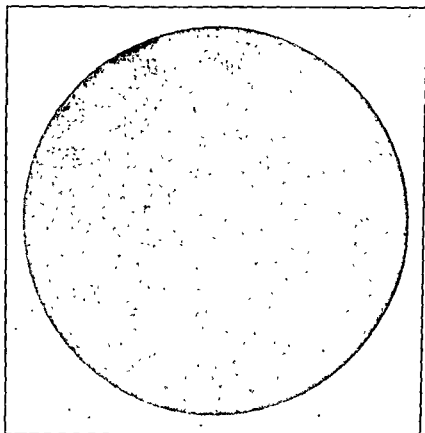
Fig. 3.—Photomicrograph of skin capillaries (X50), showing open capillaries. These can be determined numerically on the basis of number for each square millimeter of skin surface.
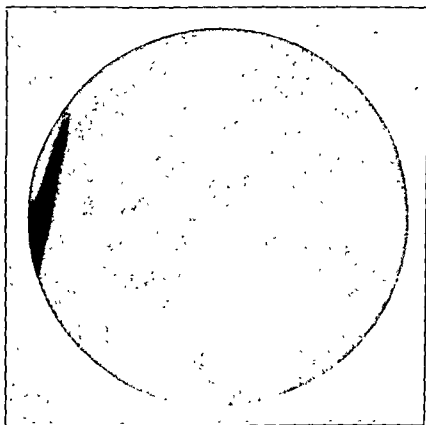
Fig. 4.—Photomicrograph. Nail-fold capillaries (X50).

ranged in parallel with the fixed resistance, so that currents of 25 to 50 amperes could be used for a few seconds when photographing. Using a total magnification of 64 (objective 16 and demonstration eyepiece 4), and

employing ordinary Eastman films, with exposure of one-tenth second, the light being concentrated upon the skin at the nail-fold by means of a suitable system of lenses (Fig. 1, c) a current of 20 to 25 amperes has proved generally satisfactory for photographic purposes. We have been able, however, using either plates or films and higher amperage, to obtain photomicrographs varying in magnification from 128 to 10 in from one-tenth to one-hundredth second.

The lantern should be angled at approximately 45 degrees with reference to the plane of the table. By means of a finger rest (Fig. 1) with its upper surface so shaped as to permit the angle of the light incident on the nail-fold to be slowly changed, the most satisfactory position and angle for photographing can be obtained through the elimination of reflections from oil surfaces and other undesirable effects.
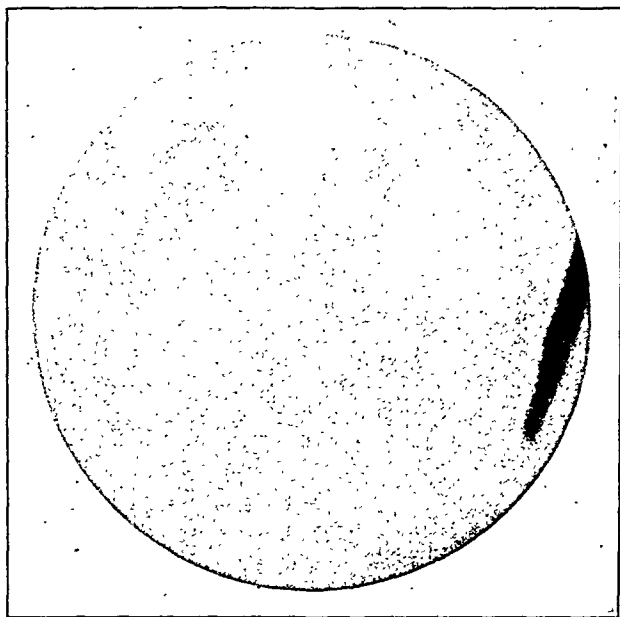
Fig. 5.—Photomicrograph. Nail-fold capillaries (X64). Case of Raynaud's disease, showing large dilated and partially filled loops, taken during the period of cyanosis.

The construction of the camera (Fig. 1, f) needs little comment. It is made to carry a No. 118 Eastman film, since this size (80 by 105 cm.) enables one to record photographically the full microscopic field in the plane which the film occupies. The use of a roll of films with six or twelve exposures enables a series of photographic records to be taken when studying the consecutive stages in certain pathologic conditions. It is, of course, essential that the operator be assured of correct focus at the photographic film at all times. This is accomplished in the apparatus which we have assembled through the use of a Leitz eyepiece (Fig. 1, d) or similar demonstration eyepiece (magnification 4 × ordinarily used), which allows the major part of the light in the barrel of the microscope to pass through the ocular and thence to the photographic film, and at the same time reflects a portion of the light to the observing eyepiece. Before entering into the routine of taking photomicrographs, there-

fore, it is necessary to determine experimentally the position of the camera in order that the image as observed on a suitable ground glass plate laid in the plane occupied by the film, may be in as sharp focus as is the image seen in the comparison eyepiece. When this position is once determined, the clamp on the rod supporting the camera is set. No further attention to this matter is required except for an occasional check up. However, the illumination obtained by the use of large currents frequently changes the depth of focus, so that refocussing with the slow adjustment screw is required during the use of the high-powered illumination.

The photographic negatives, cut to correct size, are inserted between two thin plates of glass in the slide holder of an ordinary short focus projection apparatus. Measurements on the calibers and lengths of the arterial and venous limbs, as imaged on the screen, are made with a millimeter scale. Knowing the total magnification due to the microscope and the projection apparatus, quantitative data can be readily obtained.

Figs. 3 to 5, inclusive, are reproductions obtained from prints made from the original negatives. These selections represent average results obtained by our methods, and are taken from a group of nearly 100 cases studied.

SUMMARY

A method is presented for the successful utilization of photomicrography in the study of the smaller vessels of the skin. Complete mobilization is rendered unnecessary because of the employment of instantaneous exposures. By suitable projection methods with known magnification, quantitative measurements on the total visible loop and capillary calibers can be obtained with a small percentage of error. Permanent records are thus obtained for filing or comparative studies.

AN IMPROVED STAINING AND DRYING PLATE*

By H. C. Sweaney, M D., Chicago, Illinois

CORPER† described an electric drying bath for staining tubercle bacillus wherein the slides were placed in a rack and allowed to steep over a layer of sand heated electrically by units composed of fine nichrome wire wound around "lavite" cores. We have used these baths continuously for a great many years, but find that in the long run they are rather expensive because the units frequently wear out and their cost is rather high. Therefore, we have been working on a model that has had for its object a reduction in price and a more general usefulness.

The heating is done by four longitudinal nichrome wire coils suspended in narrow openings in a transite base. These heating coils are far enough below the tray to prevent overheating and at the same time give ample circulation

of air to furnish an even temperature averaging 110° C. at the level of the slide tray.

An added feature to the older apparatus is the arrangement for drying slides at a lower temperature thus obviating any chance of error in the use of blotting paper which is sometimes (too often in fact) used for that purpose.

The detailed description follows.

110 Volt A. C. or D. C. Current
Dimension over all:
Length 16″   Width 8½″   Height 6¼″
Weight app. 14 lb.
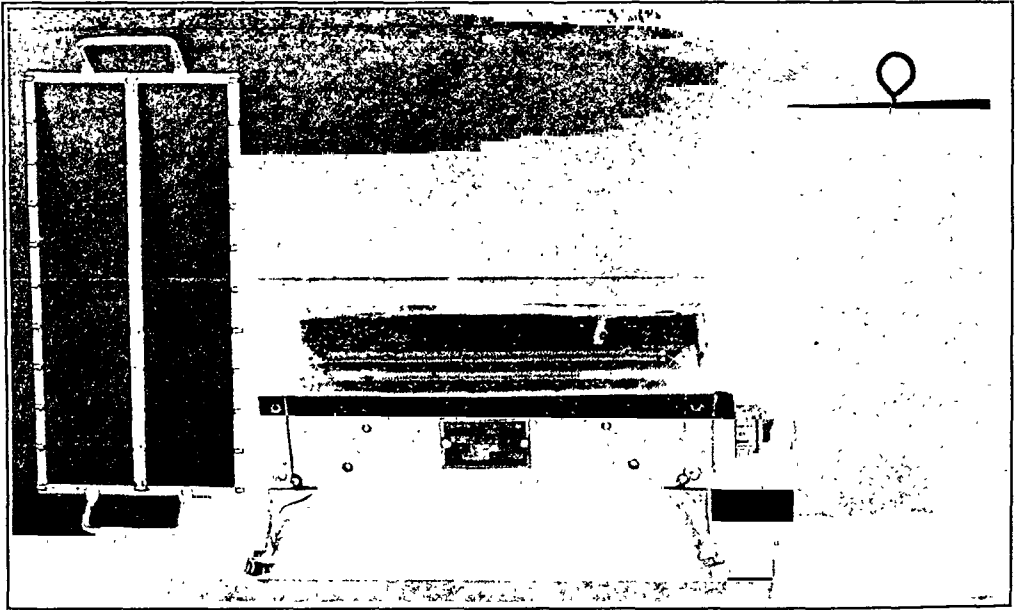
Fig. 1.

This apparatus, made entirely of aluminum, transite lined, has been designed for staining and drying bacteriologic slides. The body contains air-circulated heating coils, regulated by a three-heat switch, by means of which the desired temperature is quickly obtained and evenly maintained for any length of time. High heat is used for carbolfuchsin staining in tuberculosis work, and either medium or low heat for drying. The aluminum plate prevents injury to the elements, as it catches any staining solution, dripping from the slides. The aluminum slide rack accommodates any number of slides up to 20, and can readily be removed from the body to wash the prepared slides in the sink. A special space is provided for each slide.

A MICROMETHOD FOR THE ENUMERATION OF MICROCOLONIES*

By F. T. Briedigan and T. M. Chang, Battle Creek, Michigan

THE usual method of making a bacterial count is by the use of the counter, which is a process of division and multiplication. However, this method becomes impracticable when it comes to counting small colonies such as those of B. acidophilus, B. bulgaricus, and similar organisms which are of microscopic dimension and cannot be detected by the unaided eye. Kopeloff (by private communication) says that the usual technic of counting can be employed if the acidophilus plates are left in the incubator four or five days instead of two as commonly practiced. While the acidophilus colonies do grow larger, to some extent, with prolonged incubation, our experience seems to indicate that their size always remains microscopic, and consequently, the usual technic is hardly applicable. The difficulty is increased if the agar medium is not absolutely clear, for detritus of any sort will cause a cloudiness in the medium and will confuse with the true acidophilus colonies when examined macroscopically. Therefore, to count microcolonies with any certainty requires a definite recognition of the colonies first, in order to exclude all the pseudocolonies made of débris in the media, and for a definite recognition the microscope is indispensable. It is evident, then, that the enumeration of microcolonies can best be done under the microscope, and it is such a method, simple enough in itself but hitherto seldom used,† that we wish to describe in the following.

THE METHOD

By this method, the microscopic field is used as the counting unit corresponding to the squares of an ordinary counting plate. Since only the low power objective is used when examining microcolonies in a Petri dish, so the low power field is the basis of the microunit. The actual area of this unit will, of course, vary somewhat with the different makes of the microscope, and also with the particular ocular and tube length used, but it can be very easily calculated in each individual case according to either one of these two methods: (1) By using a stage micrometer (Hausser's), the diameter of the field can be directly read and the area calculated according to the formula $(a = \pi r^2)$, or (2) if a stage micrometer is not available, a Zeiss counting chamber can be substituted. The checkered square of the chamber (Thoma ruling) is one sq. mm. and will not entirely cover the field, therefore, a fine hair must be inserted into the ocular to serve as a pointer, dividing the diameter across the field into two sections which can then be easily measured one after

the other with the rulings on the chamber. If the second method is used, care must be taken to see that the median line of the checkered square exactly coincides with the diameter of the field. This can be ascertained by sliding the chamber to one side with the aid of a mechanical stage until the two corners of the square touch the circumference of the field simultaneously. When this position is reached, the median line of the chamber exactly coincides with the diameter of the field. Then slide the chamber on still farther in the same direction as before until the end point of the median line touches the circumference of the field, making the first vertical line of the ruling tangent to the circumference. Thus, the distance from the end point of the median line to where the pointer touches or intersects it can be measured by the division of the counting chamber. The distance from the pointer to the other end of the diameter can be calculated in the same way, and the sum of the two gives the diameter across the field; the area can be worked out in the usual way.

In the same manner the inside area of the Petri dish is measured. The area of the dish divided by the unit gives the factor which is the number of units contained in the whole dish. With this factor the counting of microcolonies is greatly facilitated. Provided the colonies are very uniformly distributed, not less than 50 units must be counted and the average taken. A tallying register will be very useful. The latter instrument will register all the colonies counted, and this divided by the number of fields observed will give the number of colonies per unit. The number of colonies per unit times the factor equals the total number of colonies in the plate, and this times the dilution will give the number of viable organisms per cubic centimeter in the original culture.

<center>REMARKS</center>

All devices for the counting of bacterial colonies are bound to have more or less technical error, and it is particularly true when a count of microcolonies is attempted. This micromethod is far from being perfect, but with reasonable caution on the part of the worker, this method can be used to minimize the wide range of probable error, which would be difficult to eliminate with any other method in the counting of microcolonies. For the best result, the bacterial suspension must be thoroughly mixed with the medium, and the mixture must be poured into the plate as uniformly distributed as possible. The units selected for the counting must be uniformly scattered all over the entire plate. If 50 units are to be counted, 25 should be at and near the periphery and the other 25 at and around the center of the plate.

Bearing all the above precautions in mind, several thousands of counts have been made with this micromethod with a view to determine the minimum units that have to be counted in order to secure a somewhat constant average per unit. Table I gives the results of 2200 counts. In the first column in the table each number represents the sum of colonies found in ten fields (10 counts). In the second column each number equals the total number of colonies per 10 units plus the preceding number in the first column which is, as above stated, also the total number of colonies per another 10 units, making

the number the sum of colonies in 20 units. Likewise, each number in the third column represents the total of colonies in 30 units, etc., etc.

TABLE I

```
—    8
    17–25
    17–30–43
    14–29–39–53
    15–26–34–44–50
     8–16–25–43–50–57
    10–22–31–36–44–50–64
    10–21–32–42–54–64–73–83
     6–12–22–30–37–47–55–67–74
     7–20–32–41–50–60–70–82–89–99
```

It will be observed that the numbers in the fifth column in Table I agree with one another more closely than the numbers in any other column. Therefore, in the routine work not less than 50 units should be counted, however, the number of units to be counted is secondary in importance. For the best results, a uniform distribution of the organisms in the medium is most essential.

To facilitate work, such dilutions of the original culture should be made as will develop not more than five or ten colonies per unit. (A culture containing 300,000,000 viable organisms per cubic centimeter is best counted by this method with a dilution of 1.10,000 and checked, if desired, by a lower dilution of 1:5,000.) More than that limit will make the counting somewhat difficult, although with the aid of a tallying register it can be done nicely.

The microscope (Spencer) used by one of us (T.M.C.) has a field radius of 0.0825 cm. when a 10X ocular and the low power objective are used. This radius gives a field area of 0.021,382,515 sq. cm. The area of an ordinary Petri dish is 65.03897 sq. cm. The latter divided by the field area gives the factor which is 3041.67. This factor has been used in counting acidophilus colonies and other similar microcolonies, and favorable results have been obtained. Table II shows the result of a counting of the viable organisms per cubic centimeter from two acidophilus cultures ·

TABLE II

Culture A	(dil:—1:1000) ... .36,436,400	per c.c.
"	(dil:—1:500) 40,080,000	" "
Culture B	(dil:—1:1000) 21,861,840	" "
"	(dil:—1:500) 17,125,108	" "

CONCLUSIONS

1. Microcolonies cannot be counted with any degree of accuracy with the usual technic under a hand magnifying lens. A micromethod is necessary for an approximate enumeration.

2. A micromethod using the microscopic field as the counting unit furnishes the worker a clear and definite recognition of the microcolonies and thus enables him to make a more reliable and comparatively more accurate count.

3. A tallying register will facilitate the work of counting greatly.

4. Provided the colonies are fairly uniformly distributed, a count of 50 fields will give a good average per unit.

SOURCES OF ERROR IN BLOOD UREA AND NITROGEN DETERMINATION*

By Elmer L. Sevringhaus, M.D., and Frances Hipple, B.S., Mendota, Wis.

IN our experience there are two sources of error in the methods for determining urea or nonprotein nitrogen in blood. Since these are frequently overlooked by technicians doing routine work, precautions are important. We have found results too high because of ammonia contained in filter paper and also in distilled water from a continuous steam heated still. The ammonia in the distilled water apparently varies, although the raw water supply is artesian from a deep stratum of sandstone. The distilled water was found to contain twice the concentration of ammonia determined for the artesian water, due to the concentrating tendency of the still. We have been using as a routine the purification of distilled water by percolation through a column of permutit, as suggested by Folin and Bell.† For this purpose we employ permutit M, supplied by the Permutit Co. at a lower price than the high grade used for most laboratory work. The only disadvantage of this process is that in the place of the traces of ammonia the water contains traces of sodium bicarbonate. Acid or alkali solutions made with this water must be standardized.

The removal of ammonia from filter paper is readily accomplished by rinsing the sheets of paper separately in ammonia-free water three times. They are dried on a pane of glass in fresh, outside air. This process was so laborious that we have searched for a brand of filter paper which could be depended on to be free from such amounts of ammonia as might interfere. For the determination of urea nitrogen by the Folin-Wu method we frequently have to use an aliquot of a 15 c.c. filtrate. The percentage error, due to contamination with ammonia, will be largest when the filtrate is small and the urea concentration low. For a 15 c.c. filtrate from a blood sample containing 10 mg. urea N per 100 c.c. a filter paper, which yields 0.0045 mg. ammonia, will cause an error of 3 + per cent. We have found only one source for filter paper which meets such a standard, using a 9 cm. paper. Eleven varieties of paper from six manufacturers were tested. Some samples showed twenty-five times this amount, which we feel is a limit of tolerance. Paper of a given variety was often variable when selected from different parts of a pack or from different shipments. The one satisfactory paper has been Durieux No. 121, a French paper distributed by the Palo Co. of New York. Numerous samples from a large shipment were found to contain 0.0048 mg. or less of N per 12.5 cm. sheet. This paper is said to be free from ammonia due to a different process from the usual one in filter paper production.

A third source of error in determining urea nitrogen is the use of too much sulphuric acid in precipitating the proteins. New technicians may do this, either due to errors in measurement or to faulty standardization of the dilute acid solution. The buffer solution prescribed by Folin and Wu is not adequate for an excess of acid.

A SIMPLE METHOD FOR THE ESTIMATION OF FREE HYDRO-CHLORIC ACID IN GASTRIC CONTENTS*

By Edward Hollander, M.D., New York City

THE Toepfer titration method is now generally employed for the quantitative determination of free hydrochloric acid in gastric contents. The amount of free acid calculated for 100 cubic centimeters of gastric contents is expressed as the number of cubic centimeters of tenth normal sodium hydroxide required to neutralize it. The indicator, dimethylamidoazobenzol, is very sensitive to free hydrochloric acid. It changes color when as little as 0.002 per cent of hydrochloric acid is present. It reacts with organic acids only if they are present in unusually large amounts (more than 0.2 per cent). In such concentration, organic acids are readily detected by their odor and by the Uffelmann test for lactic acid, so that their reaction with dimethylamidoazobenzol can be differentiated from that given by free hydrochloric acid.

The true acidity (or hydrogen-ion concentration) of a fluid can be determined accurately only by electrometric methods. As emphasized by Shohl and King,[1] in gastric contents, in which buffers are present, titration methods involve varying degrees of error. These depend upon the hydrogen-ion range of the indicator that is used and upon the buffer action of the fluid to be examined. However, in clinical work a high degree of accuracy is not necessary in the determination of the acidity of gastric contents. Considerable variations in acidity are found in the same individual in repeated test meals on different days. Such variations are due to uncontrollable psychic, sensory, and motor stimuli which reflexly affect the gastric secretion. Most clinicians are now agreed that the only value in estimating the free acidity of a test meal is to determine whether it is within the normal range, or whether hyperacidity, hypoacidity, or anacidity is present. The determination of absolute values for gastric acidity is without practical importance.

In fractional test meals, the exact determination of the acidity is likewise not important. Recent critical studies by Gorham,[2] Kopeloff,[3] and White[4] have shown that in accord with the physiologic experiments of Cannon, Gruntzler and others, the gastric chyme after a test meal is not a homogeneous mixture. The acid values of small aspirated fractions do not represent the acid concentration of the entire stomach contents.

Furthermore, the routine determination of the total acidity of gastric contents gives no definite information. The total acidity is the sum, chiefly, of the free and of the combined hydrochloric acid. The amount of organic acids and acid salts is negligible in cases in which free hydrochloric acid is present. A marked increase over the normal total acidity may be due to several conditions which are grossly evident. Thus, the hydrochloric acid may be combined with blood serum in gastric bleeding, or with duodenal fluid in biliary regurgitation, or with excessive amounts of mucus of respiratory or alimentary origin.

For the reasons given, I have employed the following simple method for the determination of free hydrochloric acid, which is sufficiently accurate for practical purposes. Dimethylamidoazobenzol has a nuance of color from light yellowish pink to deep crimson, depending not so much upon the

TABLE I

COMPARISONS OF THE FREE HYDROCHLORIC ACID IN GASTRIC CONTENTS DETERMINED BY THE
TOEPFER METHOD AND BY THE AUTHOR'S METHOD

CASE	TOEPFER TITRATION METHOD	TEST PAPER AND COLOR CHART
1	39 c.c. N/10 NaOH	40 c.c. N/10 NaOH
2	47 "	45 "
3	54 "	60 "
4	10 "	10 "
5	92 "	90 "
6	26 "	25 "
7	15 "	15 "
8	36 "	35 "
9	77 "	80 "
10	40 "	35 "
11	42 "	35 "
12	34 "	35 "
13	5 "	5 "
14	56 "	60 "
15	21 "	20 "

1) In the Toepfer Titration Method 10 c.c. of gastric contents filtered through gauze was used for each determination.

2) In the use of the Color Chart, when the color of the test paper lies between two colors of the chart, 5 is added to the value of the lighter color.

3) The slight variations in these comparative tests are negligible in test meal determinations.

amount of the indicator present as upon the degree of the hydrogen-ion concentration of the solution. Bibulous paper is dipped in an alcoholic solution of 0.25 per cent of dimethylamidoazobenzol and dried. This test paper when dipped into weak solutions of hydrochloric acid gives excellent shades of color. The range of color obtained is much greater than that given by congo paper. Five colors were selected representing acidities of 10, 20, 30, 40, 50 cubic centimeters of tenth normal hydrochloric acid per 100 cubic centimeters of water. These colors have been made into a chart similar to a Tallqvist hemoglobin scale.

Procedure.—One end of the indicator paper is dipped into the gastric contents and the resulting color is matched on the chart. It is important to match the paper immediately, before any evaporation occurs. The examination should be made in daylight against a white background. The degree

of the acidity is read directly. If the paper matches the deepest color (acidity of 50 or more), the gastric contents is diluted with an equal volume of water and the new reading is multiplied by two.

This method is accurate for practical purposes. Its chief advantage is its simplicity. It obviates the use of reagents, burettes, measuring glasses and other apparatus. The general practitioner, who only occasionally has to perform a gastric analysis, usually lacks the necessary materials. In an active clinic or a busy office where many test meals must be examined in a morning, the titration method becomes time consuming. Also, not infrequently, only a few cubic centimeters of gastric contents are obtained in the lumen of the stomach tube, a quantity too small for titration, but sufficient for examination by this method.

REFERENCES

[1]Shohl, Alfred, and King, John: The Colorimetric Determination of the True Acidity of Gastric Contents, Bull. Johns Hopkins Hosp., July, 1922, xxxi, 152.

[2]Gorham, F. D.: Variations of Acid Concentration in Different Portions of Gastric Chyme and Its Relation to Different Methods of Gastric Analysis, Arch. Int. Med., April, 1921, xxvii, 434.

[3]Kopeloff, Nicholas: Individual Variations as Influencing Rehfuss Fractional Method of Gastric Analysis, Jour. Am. Med. Assn., Feb. 11, 1922, lxxviii, 404.

[4]White, Franklin, W.: Simultaneous Variations in Acidity of Different Portions of Gastric Contents, Jour. Am. Med. Assn., October 28, 1922, lxxix, 1499.

TECHNICAL IMPROVEMENTS IN UREA DISTILLATION BY FOLIN-WU METHOD

By H. E. Butka, M.D., and F. E. Meisner, Los Angeles, California

SINCE the introduction of the Folin and Wu method of determination of urea in the blood, many technical improvements have been devised to simplify the technic of carrying out the test.

Our experiences with the various methods proposed have led us to develop a method which utilizes the good points of several of those already proposed together with a few minor modifications of our own. This method has been in use in our laboratories for the past year with exceptionally satisfactory results.

The accompanying photograph illustrates the instrument as used in our laboratory. We would call attention to the important points. First, the use of an ebullition tube, consisting of a short length of glass tubing sealed off at one end and inverted into the distilling mixture.

Second, the small capillary pipette which controls the rate of admission of air.

Third, the water jacket, which condenses the vapor and keeps the solution and gas coming in contact with the acid solution cool.

Fourth, capillary outlet for gases into the acid solution.

Fifth, aspiration by means of a small filter pump attached to any water outlet.

Sixth, apparatus held by a single clamp attached to water jacket.

In operation, the aspirator is turned on until bubbles appear in both tubes. Distillation proceeds until only a few drops remain in first tube.

OUTLET OF WATER JACKET

AIR INLET WITH CAPILLARY TUBE ABOUT $\frac{1}{10}$ MM BORE AT TIP

CLAMP TO STAND HERE ONLY

EBULLITION TUBE

DISTILLING MIXTURE

INLET TO WATER JACKET

TO ASPIRATOR

MICRO-BURNER

CAPILLARY TUBE 1 MM BORE AT TIP

ACID SOL
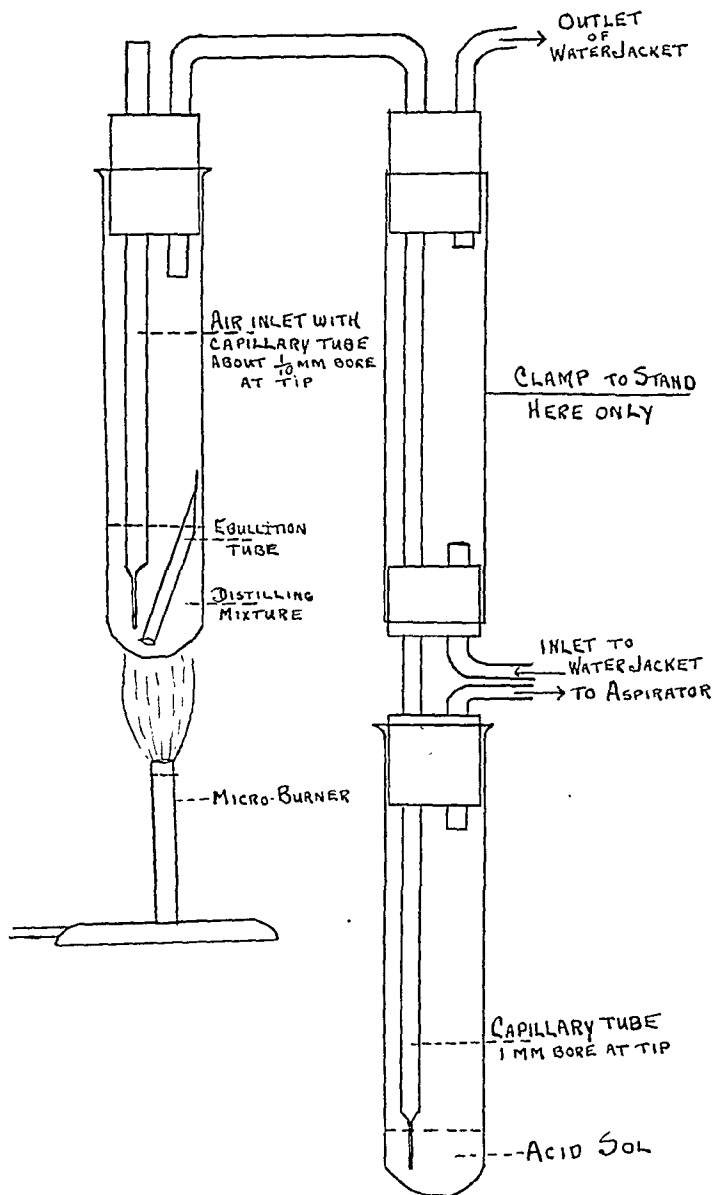
Fig. 1.

There is absolutely no back aspiration of fluid, and instrument requires no special attention during the process.

REFERENCES

[1]Folin and Wu.: A System of Blood Analysis, Jour. Biol. Chem., xxxviii, 81-112.
[2]Moore, J. W., and Jones, L.: JOUR. LAB. AND CLIN. MED., September, 1922, vii, 756.
[3]Johnson, S. Lloyd: JOUR. LAB. AND CLIN. MED., September, 1924, ix, 860., No. 12.

TRANSACTIONS

TRANSACTIONS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, FOURTH ANNUAL CONVENTION, PHILADELPHIA, MAY, 1925

THE proceedings were held in the ballroom of the Benjamin Franklin Hotel, Philadelphia, Pennsylvania, May 20 to 23, 1925.

The convention was called to order by President John A. Kolmer. The minutes of the previous meeting having been published, their reading was dispensed with. The President appointed the following members for the nominating committee: Dr. Robert A. Kilduffe, Dr. Harry J. Corper, Dr. Wm. C. MacCarty. No other business being transacted at the morning session the time was devoted to the reading of papers on the regular scientific program as follows:

The Preparation of Solutions of Dextrose for Intravenous Administration, by Dr. Ralph G. Stillman, New York City, N. Y. This paper was discussed by Dr. F. M. Huntoon and Dr. John A. Kolmer.

Utilization of Carbohydrates, by Dr. W. G. Karr, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. Wm. G. Exton, Dr. Herman Sharlit, Dr. Frank W. Hartman, Dr. John A. Kolmer. Discussion closed by Dr. W. G. Karr.

Diagnostic Value of Spinal Fluid Sugar Content, by Dr. W. Parker Stowe, Rochester, N. Y. Discussed by Dr. John A. Kolmer, Dr. Ward Burdick, Dr. A. S. Giordano. Discussion closed by Dr. Stowe.

Alveolar CO_2 Tension and Acetone in the Expired Air in Acidosis, by Dr. Paul Roth, Battle Creek, Michigan. Discussed by Dr. A. Z. Wolodarsky, Dr. W. Parker Stowe, Dr. Frank W. Hartman. Discussion closed by Dr. Roth.

The Effect of Varied Carbohydrate Food in the Blood Sugar Concentration, by Dr. Leon Jonas, Philadelphia, Pa. (By invitation.) No discussion.

<center>WEDNESDAY, MAY 20, 2 P. M.</center>

President John A. Kolmer called the meeting to order and the scientific program was continued.

Aids to Urinalysis in a Large Hospital, by Dr. C. Pons and Dr. E. B. Krumbhaar, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. Wm. G. Exton who showed a slide illustrating his method and Dr. Henry Stewart. Discussion closed by Dr. Krumbhaar.

A New System of Duplicating and Placing Laboratory Reports in the History, by Dr. Frank W. Hartman, Detroit, Michigan. Discussed by Dr. E. B. Krumbhaar. Discussion closed by Dr. Hartman.

Value of Method of Keeping Records of Tissue Examinations, by Dr. L. A. Turley, Norman, Oklahoma. Discussed by Dr. Robert A. Keilty, Dr. Ralph G. Stillman, Dr. A. H. Schade, Dr. James B. Bullitt, Dr. Frank W. Hartman, Dr. Leon S. Lippencott. Discussion closed by Dr. L. A. Turley.

Value of the Leucocyte Fragility Test in the Prognosis of Pneumonia, by Dr. C. Pons and Dr. E. P. Ward, Philadelphia, Pennsylvania. (By invitation.) Paper read by Dr. E. B. Krumbhaar. No discussion.

A Plea for a Standard Method of Determining and Reporting Hemoglobin Values, by Dr. E. Clarence Rice, Jr., Washington, D. C. Discussed by Dr. Stoner, Dr. Carl Spohr, Dr. E. B. Krumbhaar, Dr. F. E. Sondern, Dr. John A. Kolmer. Discussion closed by Dr. Rice.

A New Type of Instrument for the Estimation of Hemoglobin, by Dr. C. E. Roderick, Battle Creek, Michigan. Read by title.

The Value of the Icteric Index in Differentiating Anemia, by Dr. A. V. St. George and Dr. A. L. Brown, New York City, N. Y. Discussed by Dr. Herman Sharlit, Dr. Ralph G. Stillman, Dr. A. V. St. George. Discussion closed by Dr. A. L. Brown.

Some Useful Apparatus, by Dr. Max Shawcker, Dover, Ohio. Discussed by Dr. Frank W. Hartman. Discussion closed by Dr. Shawcker.

Clinical Classification of Cardiac Defects, by Dr. Maude E. Abbott, Philadelphia, Pennsylvania. No discussion.

The Physiology of the Circulation in Certain Congenital Cardiac Defects, by Dr. B. T. Dawson. No discussion.

THURSDAY, MAY 21, 9 A. M.

The meeting was called to order by President Kolmer and the scientific program was continued.

A Comparison of Enzyme Actions of Tumors and Normal Tissues, by Dr. K. George Falk and Helen Miller Noyes, New York City, N. Y. (By invitation.) Discussed by Dr. John A. Kolmer, Dr. Wm. G. Exton. Discussion closed by Dr. Falk.

Some Enzyme Studies with Desiccated Gonadal Tissue, by Dr. Herman Sharlit and Dr. William G. Lyle, New York City, N. Y. Discussed by Dr. K. George Falk, Dr. John A. Kolmer, Dr. H. J. Corper. Discussion closed by Dr. Sharlit.

A Double Hydrogen Electrode System for the Determination of Hydrogen-Ion Concentration, by Dr. George H. Meeker and Mr. Bernard Oser, Philadelphia, Pennsylvania. (By invitation.) No discussion.

Observations on the Dick Test, Toxin Immunization and Serum Treatment of Scarlet Fever, by Dr. John A. Murphy, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. Kolmer, Dr. F. M. Huntoon, Dr. Herman Sharlit, Dr. E. Clarence Rice, Dr. H. A. Heise, Dr. David Bergey. Discussion closed by Dr. Murphy.

Pneumococcus Antibody Solution, by Dr. Frank M. Huntoon, Glenolden, Pennsylvania. Discussed by Dr. Wm. G. Exton, Dr. A. S. Brumbaugh, Dr. Carl Spohr, Dr. R. A. Kilduffe, Dr. A. C. Abbott, Dr. David Bergey, Dr. Robert A. Keilty. Discussion closed by Dr. Huntoon.

Autopsy Report of Two Cases of Thymic Death During Surgical Operations, by Dr. Jessie W. Fisher, Middletown, Connecticut. Read by title.

AFTERNOON SESSION, MAY 21, 2 P. M.

Meeting called to order by President Kolmer, scientific program continued.

Normal and Pathologic Basal Metabolic Rate in Obesity, by Dr. Horry M. Jones, Chicago, Illinois. No discussion.

Studies on Blood Cultures with a Special Reference to the "Massive Method," by Dr. Herbert Fox and Dr. William G. Leaman, Philadelphia, Pennsylvania. Discussed by Dr. A. I. Rubenstone, Dr. John G. Wurtz, Dr. F. M. Huntoon, Dr. Leon S. Lippencott, Dr. H. A. Heise, Dr. R. A. Kilduffe. Discussion closed by Dr. Leaman.

A Comparative Study of Liver Functional Tests, by Dr. A. I. Rubenstone and Dr. Louis Tuft, Philadelphia, Pennsylvania. Discussed by Dr. Ralph G. Stillman.

Studies on the Bacteriology of the Urine in Cooperation with Catheterization of the Ureters, by Dr. Robert A. Keilty, Danville, Pennsylvania. Discussed by Dr. A. H. Sanford, Dr. A. I. Rubenstone, Dr. Frank W. Hartman, Dr. H. J. Corper, Dr. A. H. Schade, Dr. A. S. Giordano. Discussion closed by Dr. Keilty.

A Study of Cases of Acute Leukemia and Acute Mononucleosis, by Dr. B. L. Crawford and Dr. Harold W. Jones, Philadelphia, Pennsylvania. Discussed by Dr. A. I. Rubenstone and Dr. W. Parker Stowe.

Blood Counts in Mississippi, by Dr. Leon S. Lippencott, Vicksburg, Mississippi. Discussed by Dr. James B. Bullitt, Dr. Ralph G. Stillman, Dr. W. Parker Stowe. Discussion closed by Dr. Lippencott.

Anisocytosis and Increased Red Blood Cell Volume with little or no Anemia, by Dr. Mortimer Warren, Portland, Maine. Discussed by Dr. Frank W. Hartman, Dr. H. A. Heise. Discussion closed by Dr. Warren.

THURSDAY, MAY 21, 7 P. M.

Meeting called to order by President Kolmer and scientific program continued.

Reaction After Typhoid Vaccination, by Dr. Henry J. Nichols, Washington, D. C. Discussed by Dr. John A. Kolmer, Dr. F. M. Huntoon, Dr. A. S. Brumbaugh, Dr. Robert Kilduffe, Dr. Robert A. Keilty. Discussion closed by Dr. Nichols.

The Measurement of Cloudiness in Liquids, by Dr. Wm. G. Exton, Newark, N. J. Discussed by Professor Jerome Alexander. Discussion closed by Dr. Exton.

Studies in Embalming Fluids in Relation to Necropsies, by Dr. John A. Kolmer and Dr. Fred Boerner, Philadelphia, Pennsylvania. Discussed by Professor Jerome Alexander, Mr. Papallo, Dr. A. S. Giordano, Dr. A. S. Brumbaugh, Dr. Margaret A. Miller, Dr. Case, Dr. T. H. Boughton, Dr. Ralph G. Stillman. Dr. John A. Kolmer closed the discussion.

The Specific Inflammatory Reaction of Immunized Animals (Arthus Phenomenon), by Dr. Eugene L. Opie, Philadelphia, Pennsylvania. (By invitation.) No discussion.

Methods of Staining Tubercle Bacilli, by Dr. H. J. Corper, Denver, Colorado. Discussed by Dr. Robert A. Keilty and Dr. A. H. Sanford. Discussion closed by Dr. Corper.

President Kolmer then called upon Dr. Maude E. Abbott who spoke on the Osler Memorial.

FRIDAY, MAY 22, 9 A. M.

Meeting was called to order by President Kolmer and scientific program continued.

Sedimentation Rate of Erythrocytes, by Dr. H. N. Cooper, Watertown, N. Y. Discussed by Dr. Ward Burdick, Dr. S. L. Luboff, Dr. R. A. Kilduffe, Dr. George Fussell, Dr. O. Lowy, Dr. M. A. Miller. Discussion closed by Dr. Cooper.

Standardization of Tuberculin, by Dr. Joseph D. Aronson, Philadelphia, Pennsylvania. (By invitation.) Discussed by Dr. John R Ruchel.

Bronchial Spirochetosis, by Dr. Thomas L. Ramsey, Toledo, Ohio. Discussed by Dr. F. H. Lamb, Dr. H. J. Nichols, Dr. O. Lowy, Dr. A. H. Schade. Discussion closed by Dr. Ramsey.

Sarcoma in Psoriasis, by Miss Mary Marcus, Philadelphia, Pa. (By invitation.) Discussed by Dr. John A. Kolmer, Dr. F. L. Burnett, Dr. Martha A. Wood, Dr. H. J. Nichols. Discussion closed by Miss Marcus.

Squamous Cell Carcinoma of the Gall Bladder, by Dr. Frank W. Hartman and Dr. W. E. Johnson, Detroit, Michigan. Discussed by Dr. H. N. Cooper, Dr. A. S. Giordano. Discussion closed by Dr. Hartman.

The Technic of the Practical Application of the Pathogen Selective Cultural Method, by Dr. A. I. Rubenstone, Philadelphia, Pa. Discussed by Dr. Herman Spitz, Dr. O. Lowy, Dr. Cohen, Dr. J. J. Moore, Dr. Stella Gardner. Discussion closed by Dr. Rubenstone.

FRIDAY, MAY 22, 2 P. M.

The society was called to order by President Kolmer. The report of the Committee on Laboratory Standardization was taken up and Dr. Ruth Gilbert was asked to discuss the plan. Dr. Sondern, at the request of President Kolmer read the proposed plan and the discussion was continued by Dr. C. Y. White, (by invitation). Dr. N. P. Colwell, Secretary of the Council on Medical Education and Hospitals of the American Medical Association next discussed the proposed plan. The question was then open for general discussion.

A motion was made that the society approve the proposition as a whole and the committee be ready to present to the business meeting a specific proposal in the matter. This motion was seconded and carried. The problem was then discussed by Dr. Wm. G. Exton, Dr. Maude E. Abbott, Dr. Ruth Gilbert, Dr. K D. Graves, Dr. H. J. Corper, Dr. J. J. Moore, Dr. R. G. Owen, Dr. Warren T. Vaughan, Dr. John G. Wurtz, Dr. L. A. Turley. The scientific program was then continued.

How Can We Best Promote the Objects Contained in Article II of Our Constitution, by Dr. Herman Spitz, Nashville, Tennessee. Discussed by Dr. Frank M. Huntoon.

Suggested Method to be Followed in Developing a Standardized Course for Medical Technicians, by Dr. Walter E. King, Detroit, Michigan. Discussed by Dr. R. A. Kilduffe, Dr. A. H. Sanford, Dr. A. S. Brumbaugh, Dr. Robert A. Keilty, Dr. F. M. Huntoon. Discussion closed by Dr. King.

FRIDAY, MAY 22, 7 P. M.

On Friday, May 22, at 7 P. M. the annual dinner was held. A sumptuous menu was served. President John A. Kolmer read his address, ''Education as a Cure for Present-Day Evils in Clinical Pathology.'' (See page 891 of this issue.) Dr. George H. Meeker, Dean of the Graduate School of Medicine, University of Pennsylvania delivered an address on the ''Functions of a Hospital.'' Dr. M. T. MacEachern, Director of Hospital Activities of the American College of Surgeons, who was also a guest of honor at the Rochester meeting presented a paper on the ''Rôle Played By the Pathologist in Hospital Service.'' (See page 898 of this issue.)

SATURDAY, MAY 23, 9 A. M.

Business Session

President Kolmer.—The first order of business is the election of new members. I will call upon the Board of Censors for their report.

Dr. A. H. Schade.—The Board of Censors has gone over and approved seventy-three applications, five being for associate membership and some of these will be active members as soon as they can qualify. We have one application here which, if it is acted upon favorably by the society, establishes a precedent. The applicant is a colored physician who comes very highly qualified, vouched for by Dr. Kolmer. The Board of Censors feels that it should be brought up separately before the society as a special application.

President Kolmer.—You have heard the report of the Board of Censors, is there any discussion of this report?

Dr. Spitz.—In regard to the application from the colored physician, I am from the South. I know one colored physician in clinical pathology who is about as competent a clinical pathologist as I know. I would be the last one to vote against the applicant. According to our rules an applicant must be a graduate in medicine and a member of his local state society. If he can qualify in these respects, I would be glad to vote for him.

Dr. O. Lowy.—I move that this applicant be elected if he can qualify, and I believe he can.

President Kolmer.—I do not know whether the Board of Censors has any data. His election this morning might be unconstitutional.

Dr. Lowy.—I understood that this applicant is qualified in every way.

Dr. Spitz.—This man is not a member of his local state society. He is a member of the Kansas State Society. I am frank to tell you that under conditions existing below the Mason-Dixon line this man cannot qualify because he cannot become a member of the county society.

Dr. Sondern.—You cannot be a member of the American Medical Association without being a member of the local society.

Dr. Lowy.—Can he not qualify for associate membership? I believe we would be doing an injustice and not furthering our own cause if we kept out competent men.

President Kolmer.—That is a thought that has occurred to me. He might become an associate member. What is the pleasure of the society?

Dr. Lowy.—I move that this man be elected to associate membership.

Dr. Spitz.—I second the motion.

Dr. Sondern.—It should be stated why he was not elected to regular membership.

Dr. Lowy.—I accept the amendment.

President Kolmer.—It has been moved and seconded that we elect this candidate to associate membership, notifying him of the reasons why he could not be elected to active membership, all in favor, aye; opposed, nay; order carried.

Dr. Spitz.—I move that all the other applicants be elected to active membership as recommended by the Board of Censors. Motion seconded and carried.

A motion was made that all applicants for associate membership be elected. Motion was seconded and carried.

President Kolmer.—I would like to ask whether graduates in veterinary medicine are eligible to the society. I have in my own laboratories two doctors of veterinary medicine who

have devoted all of their professional career to the laboratory. The training that they have had in veterinary medicine has been a very good background. I would like to ask an expression of opinion as to this matter.

Dr. Spitz.—It seems to me that if they are qualified in bacteriology, they are scientific men. Section three of the constitution reads: "Associate members shall be graduates of recognized scientific institutions, who have made valuable contributions to any of the sciences relating to Clinical Pathology, or whose association with this organization will further the objects of this society. . . ." It seems to me that under that clause the two men mentioned are contributing to the welfare of clinical pathology and I should think would be eligible for associate membership in the society.

President Kolmer.—I am going to interrupt the regular order of business in order to receive the report of the Committee on Standardization of Laboratory Reagents of which Dr. Huntoon is chairman.

Dr. Huntoon.—The Committee on the Standardization of Laboratory Reagents submitted a report at the San Francisco meeting and this was published in the proceedings. It must have been satisfactory because I did not receive a single complaint. Nevertheless, the committee has been inactive for the past year because there was no further activity in that particular line; it was only by criticism from the members that we could change the standards set up. I feel that the time has arrived when the committee should be reorganized, not for establishment of standards, but for establishment of approved methods. I want to resign as chairman of this committee. I am not in a position to help with these other things. Someone who is in very active clinical pathologic practice should be the chairman of such a committee.

President Kolmer.—You have heard the report of the Committee on Standardization of Laboratory Reagents. Are there any comments?

Dr. Lowy.—I move that we adopt this report and the recommendations be accepted.

Dr. Kilduffe.—I second the motion.

The motion was carried.

President Kolmer.—We will now proceed with the regular order and ask for unfinished business.

Dr. Burdick.—At the last meeting the matter of creating the office of president-elect was brought up. It may now come up for a vote.

Dr. Spitz.—I believe that the object in that proposal last year was to follow in harmony with other organizations who have created the office of president-elect rather than first vice-president. I believe it would be a good thing to adopt. I, therefore, move that we change the clause in our constitution that we elect a president-elect instead of a first vice-president.

Dr. Burdick.—The clause as it stands submitted is to elect each year a president-elect and a vice-president in place of the present custom of electing a first vice-president and a second vice-president. This motion has been made; is the amendment acceptable to Dr. Spitz?

Dr. Spitz.—This is acceptable.

President Kolmer.—All in favor of this motion say aye, contrary nay, so ordered.

Under unfinished business I will also ask the Executive Committee to submit its report on the examination of the books.

Dr. Spitz.—The Executive Committee examined the books of the treasurer and the vouchers. We have found that the books tally with the bank. There is a statement from the bank showing a balance of $723.48 to the credit of the society. There were two items of expenditure that amounted to a considerable amount, that involved the expense of our secretary to the College of Surgeons Convention. These two trips were undertaken by our secretary with the consent of the Executive Board and we feel that the thanks of the society are due Dr. Burdick for several reasons: first, for his painstaking care in getting up papers to be read before the American College of Surgeons presenting the attitude of our society along certain lines, thereby gaining the complete cooperation of the College of Surgeons as expressed last night by Dr. M. T. MacEachern; then we owe our thanks to Dr. Burdick for the sacrifices of the valuable time that it takes, not only for such articles and papers, but for

the time he has devoted to the welfare of the society. I move that this society give a rising vote of thanks to Dr. Burdick.

President Kolmer.—You have heard the report of the auditing committee and the motion that this society, by a rising vote of thanks, extend to our secretary our appreciation of the time and valuable service he has rendered to the society.

Dr. Burdick.—I want not only to state my appreciation of your expression, but I wish to tell you that all of these efforts are shared by the so-called Denver committee, Drs. Corper and Hillkowitz, who have entered very heartily into all these activities and have given me their support to carry these things along. (Applause.)

President Kolmer.—If it is in order I would like to have a report on the financial status of the commercial exhibits in connection with the convention this year, either by the secretary or by Dr. Duck.

Dr. Burdick.—Dr. Duck feels that it is not an opportune time to make the report. He can make the report to me and we can announce it at the next convention.

President Kolmer.—We will now continue with the reports of committees. I will ask Dr. Corper to report on the Publication Committee.

The report of the Publication Committee by Dr. Philip Hillkowitz was then read by Dr. H. J. Corper.

REPORT OF THE COMMITTEE ON PUBLICATION

To the Officers and Members of the American Society of Clinical Pathologists:

We submit herewith a report of the activities of the committee on publication since our last meeting at Rochester. Some time prior to the Rochester Convention, tentative arrangements had been made with the publishers of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE to print the papers read at our scientific sessions.

During the meeting at Rochester the committee conferred with the editor of this journal and closer relations were established with the result that the JOURNAL OF LABORATORY AND CLINICAL MEDICINE became the official organ of the society. A substantial reduction in the subscription rate was made in favor of members of the society.

The scientific papers read at the Rochester meeting have been printed in full in successive issues of the periodical. A detailed report of the proceedings, together with the addresses of invited guests, have likewise appeared in its columns. A special department devoted to news items and official announcements of our organization has been placed at our disposal.

We desire to utilize this occasion in giving public expression of our appreciation of the courtesies shown the committee and the society by The C. V. Mosby Co., the publishers, and Warren T. Vaughan, editor, who have at all times shown a willingness to meet the requests made on them.

We are likewise grateful to them for the abundant space allowed us in the Convention Number, giving a history of the society and the program of the convention.

The question of issuing our own publication has been frequently discussed by the members of this committee, both in person at the conventions as well as by correspondence.

It cannot be gainsaid that the JOURNAL OF LABORATORY AND CLINICAL MEDICINE does not in all respects fulfill the ideal of an official organ devoted exclusively to clinical pathology. Objection has been made to the admixture of subject-matter which, though thoroughly scientific, yet is of interest only to a rather limited class of pharmacologists and of no particular appeal to members of our society. The lack of an Abstract Department, giving a review of current literature in the various branches of our specialty, has also been a source of complaint. We leave out of account the rather modest and backstage position our participation in the journal shows in its make-up, although most of our members, from a natural feeling of pride in their society, would like to see us occupy a more conspicuous place.

On the other hand, the arguments for publishing our own journal are met with the cold and cruel facts that it requires money as well as a considerable expenditure of time, energy and talent which, alas, in the present young stage of our organization, are lacking. All these worries are now vicariously carried by the JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

The committee, therefore, deems it advisable for the present to continue the arrangement as before. The harmonious relations existing between the publishers of the journal and the society encourage us into the belief that such reforms as above outlined may be incorporated in the journal with benefit to its subscribers in increased usefulness and with added prestige to the American Society of Clinical Pathologists.

PHILIP HILLKOWITZ, M.D., *Chairman.*

President Kolmer.—You have heard this report of the Publication Committee. What is your pleasure regarding it? It has been moved and seconded that this report be accepted, is there any discussion?

Dr. Sondern.—I have heard from time to time that the publishers of this journal feel that it has just begun to serve its real function and would be useful, not only to the readers, but to the owners as well. I believe that I speak correctly that they appreciate what this society has done to benefit the journal. I believe a little diplomacy between our society and those who control the journal will lead to a better arrangement. I would like to suggest that the Publication Committee use its best efforts to bring about a change in the location of the table of contents. I do not know of any of the members that understand the matter better than the committee.

President Kolmer.—Are there further comments?

Dr. Corper.—I am just informed by Dr. Exton that they have consented to make one important change that we are glad to hear about. The table of contents is going to be changed and put on one page in a better place.

President Kolmer.—I hope the Publication Committee will ask if it cannot be printed on the back page of the journal.

Dr. Exton.—We have been working towards this.

President Kolmer.—All in favor of accepting this report please say aye, contrary nay, so ordered.

I think Dr. Spitz has referred to the Executive Committee a matter relating to the method of transmitting specimens.

Dr. Spitz.—At the last meeting, at the suggestion of several of the members, first among whom was Dr. Burnett, I presented a proposition that the society issue a small leaflet in which should be printed specific instructions on how to transmit or submit specimens of various kinds to laboratories for examination. Some concrete examples of the wrong way to submit specimens to the laboratory were submitted by one of the members yesterday. This leaflet was to be issued by the society and through the various members be reissued to the general practitioners. This matter was left in the hands of the Executive Committee and referred to Dr. Stone. We regret Dr. Stone is not here. We have corresponded with him, but have not come to any conclusions about the pamphlet. I thought it would be rather presumptuous on my part to go ahead with it. Thinking of the work Dr. Sondern was doing with his committee, it occurred to me that the work might interlap, therefore, we would ask for further time so that the two committees might get together on the proposition.

President Kolmer.—Have you a motion to make?

Dr. Spitz.—I move that the Executive Committee be granted further time.

The motion was seconded.

Dr. Sondern.—The United States Postal Laws state that any specimen that contains materials that have to do with communicable diseases cannot be transported through the mails except upon receipt of a special permit from the postal authorities to receive this package. In recent applications for approval of laboratories it is stated that the applicant should, or must have a permit of this kind. Now this was put into the laws some years ago and for some time, at irregular intervals, I made application to the Post Office Department for a permit of that kind and sometimes it was acknowledged and sometimes it was not, but I never received the permit. When Dr Hubert Work became Postmaster-General, I applied for this permit and received it in forty-eight hours. As the postal laws call for this, it might easily become one of the functions of the society. It has to do with this very committee and I think some communication should be had with the Postmaster-General's office so that it might not be so difficult to obtain these specimens that we do receive by mail. This committee might do something to help along this line.

President Kolmer.—Would that have to be a general ruling from the Postmaster-General's office?

Dr. Sondern.—It is a permit that is issued in Washington; it comes through with the mail. Many laboratories lack this permit and the material is delivered to them just the same. I think we all want to try to live up to the law.

Dr. Exton.—It would be a good thing to clear this up, I second the motion.

Dr. Cooper.—In order to obtain that approval it was first necessary to get the permit from the general post office; it took only about two weeks to get the approval.

President Kolmer.—It is a very important question. Everything may work along all right for years, then something may go wrong. I will take it upon myself to request the chairman of that committee to look into the matter.

Dr. Sondern.—Under this same law these specimens have to be packed in a specific way; these rules should be made in this leaflet as well. It is an outrage the way we sometimes receive specimens by mail. Specimens of tuberculous sputum are received in terrible condition.

President Kolmer.—It is really a matter of first rate importance. The motion, I think is understood now with the two amendments and the amendments accepted. All in favor say aye; contrary, nay; so ordered. Before proceeding with the business in order, an additional application for membership has been made and endorsed by the Board of Censors. Is it your wish that we accept this applicant? All in favor say aye; contrary, nay; so carried. We now come to the report of the committee on Laboratory Standardization, Dr. Sondern.

Dr. Sondern.—Your committee has taken into consideration every criticism and every suggestion when the subject was discussed yesterday and I think that the members of the committee are in accord with the one very good suggestion that this resolution be presented and that a good deal of discretion be allowed the committee. Therefore, I have abbreviated the report that was printed.

President Kolmer.—When you have heard this report of the Committee on Standardization of Laboratories, I will entertain a motion.

Dr. Stillman.—I move that this report be accepted and time extended to the committee. Seconded.

Dr. Lowy.—May I suggest that the doctor be licensed in the state where he is practising?

Dr. Exton.—I certainly think this committee has gone into the matter in the right way. The report is a very fine and practical one. I would like to add an amendment to it whereby the committee be empowered to confer with similar committees of the American Medical Associaiton and the American College of Surgeons and any other associations that are interseted in the subject of clinical pathology.

President Kolmer.—Is there further discussion? There are two amendments.

A voice.—I would like to know how this might apply to hospital laboratories.

Dr. Sondern.—I believe that this system of approval should apply to any laboratory. A hospital laboratory may be different from a private laboratory, but practically I know of no hospital laboratory that does not undertake the same functions as a private laboratory. You can submit a specimen of any kind to any hospital laboratory that I know of and receive a report just as from any other laboratory. That being so, I do not see why a hospital laboratory should not comply as well as the other laboratories.

Dr. Spitz.—That point is so intricate I think we ought to discuss it somewhat. Take my situation; my private laboratory is situated in an office building just one block from the hospital where I supervise the laboratory work. The technicians in each laboratory are equally competent. I divide my time between the two laboratories. Would I be required to be inspected twice and would I be required to pay two fees for the examination? I know of one clinical pathologist who lives in New York City who looks after three hospital laboratories. In that case would that man be expected to undergo three examinations in addition to his private laboratory and would he be expected to pay three fees? If I am a good pathologist in my private laboratory, why should I not be equally competent in my hospital laboratory a block away? It seems to me that one examination should suffice for both.

Dr. Lamb.—I have five laboratories. I can say further that no one of them is complete; taken all together they are complete. For instance, all the tissue work for the five hospitals is done at one hospital; no one hospital is fully equipped. Should the examination apply primarily to the laboratory or primarily to the man who is responsible?

Dr. Hartman.—This is a very important question. We are not trying to standardize only the private laboratories. I do not think that is of as much interest to us or to members. It should point more to the hospital laboratory than the private laboratory. Dr. MacEachern brought out this idea; he thought that individual laboratories scattered through the hospital were not under competent supervision. The committee apparently thinks we should not go too far in the idea of letting a man spread himself out to too many laboratories or too many hospitals.

Dr. Giordano.—I am in somewhat the same situation. I work in two hospital laboratories and I am busy enough in looking after two. As it is, it is very difficult to be in both places. There should be a limit as to how many hospitals a man can handle. One should be licensed in the state where one is practising, but so far I have not applied for a license in Indiana. There should be a time limit of one year to get a license.

Dr. Kilduffc.—While it is very intricate and very complicated we ought to avoid what Dr. Huntoon mentioned yesterday. As I understand the proposal of this endeavor to survey the laboratory situation, it was to survey it as a whole. I do not think we should go forth primarily to standardize the hospital situation or primarily to improve the private laboratory; our aims should be an approval of laboratories in general. They should include all laboratories which are engaged in the diagnosis, treatment and management of disease. It is not going to be settled in two or three minutes. It is a very difficult situation to see how laboratory activities can be spread over a tremendous territory and be correctly attended to everywhere. Every laboratory should have a certain minimum amount of equipment.

Dr. Miller.—I have a friend who is licensed in one state, but she has recently moved to another state. She is not going to stay more than a year or two and does not want to pay the reciprocity fee for that short time. In the meantime she is fully qualified to do all types of work which she is handling. At the same time, her laboratory would not be approved because she was not approved. It seems to me that some technical point might be passed over in such a case.

Dr. Exton.—It seems to me that the fact has been lost sight of that this report gives merely basic recommendations that are considered worth while in the matter of standardization. The idea of the standardization is to give good service and the committee that will have charge of this can be expected to use wisdom and discretion. I do not think there was any attempt made in this report to put hard and fast rules to our laboratories.

Dr. Sondern.—I do not think that it is possible to make any hard and fast rules. I think that when the committee functions it will have to take into consideration merits of each individual case. Where a man has charge of several laboratories I do not think that the inspection or whatever is decided on, should be considered as a separate examination; but I do think that all the laboratories with which he is connected should be inspected and then all considered one proposition. Some laboratories are extensive, others very simple. I think all this must necessarily be left to the committee that will have this matter in charge.

Dr. Spitz.—I have the utmost confidence in the integrity of this committee. I raised the question at the request of several members so that we could get a public clarification of that point. Some were a little bit dubious of the examinations. I am fully aware of the purpose of the committee and in addition to everything that has been said, I want to remind the members that this proposition is voluntary under the present system.

Dr. Martin.—It is very important that some such standardization may be had so that laboratories and men in charge that are not so qualified may be pointed out.

President Kolmer.—There is one additional point; that is the requirement that the applicant for a certificate of approval must be a graduate in medicine. What is the status of the Ph. D.?

Dr. Sondern.—The committee did not take this into consideration at all because up to the present the approval of laboratories is to be confined to the membership of this association. Now as everyone who is a member of this society must be a graduate in medicine,

we did not take into consideration the laboratories run by nonmedical graduates. I know of a number of splendid clinical pathologists that are not graduates in medicine. I would suggest that we leave that to further development and the many questions that will arise that your committee will have to deal with. They will have to come to you often for advice and counsel and recommendation and resolutions on the many, many questions that do not occur to us now. I would like to see this thing started and next year the committee will have quite an elaborate report for you.

President Kolmer.—We may now place the report for action. There have been, however, two amendments, one stating that the applicant for approval shall be licensed.

Dr. Lowy.—I think that this was discussed here yesterday, but since the committee has seen fit to reintroduce the question of licensing, they should further confine it by stating where the applicant should be qualified.

President Kolmer.—I presume that this is one of the matters that we will leave to the committee. I believe it was Dr. Exton who also offered an amendment to the motion that this committee be also authorized to confer and cooperate with the officers of other organizations engaged in similar endeavors, among which the American Medical Association, the American College of Surgeons, and the American Public Health Association were mentioned. Might we incorporate this as an amendment or may we leave it to the committee? I think we may very easily leave this to the committee. The report of this committee stands as presented. All in favor of accepting this report will please say aye; contrary, nay; so ordered.

Now I believe this brings us to new business. It has been suggested under new business that a committee be appointed to cooperate with a similar committee from the American Roentgenologic Society for the adjustment of problems common to both.

Dr. Sondern.—The association of the x-ray laboratory and the clinical pathologist came about when both of these specialties were young. I remember well, years ago when radiology first came in, I thought that it was going to be one of the functions of the clinical pathologist. As a matter of fact, I succeeded when I was in Berlin in actually bringing the first x-ray machine to America. We as clinical pathologists are no longer associated more closely with the roentgenologist than we are with any others and I do not think that in this modern day there is any need for any association with roentgenologists to further the purposes of this society. I would like to express my disapproval of complicating our functions in any such manner as has been proposed.

Dr. Exton.—I move it be tabled.

President Kolmer.—Is there any further discussion? I will take the suggestion that has been made and place the matter on the table. It has also been suggested that a committee be appointed to consider the matter of standardization of laboratory procedures. I think this is a splendid suggestion.

Dr. Burdick.—That was suggested to me by a member and I told him I would call your attention to it.

Dr. Spitz.—As I suggested in my paper, the third object in Article II is to establish uniform standards in laboratory work. I do not think that uniform standards are desirable. If we say that a certain method is better than others we remove the incentive to further research according to the second object of Article II, and I suggest the term optional standards. We may have more than one procedure that may give equal results. We can arrive at the same result by two or three different methods. I think the term optional standards should be used and two or three methods that are desirable, available and accurate be allowed to be used. Personally I prefer the Ziehl-Neelsen method for tuberculosis. I think I can find tubercle bacilli by my method just as quickly. These other methods are equally as good. It should be left to the individual as to what method he prefers.

Dr. Exton.—I would, therefore, suggest that the Committee of Reagents as it is going to be reorganized, deal with the whole question of laboratory methods, including reagents, and I think Dr. Spitz's point was very well taken. Approved methods are what we have in mind. That would cover this optional idea. This committee's functions should be broad enough to include apparatus. Some day the society might publish a book having all of its approved methods. I, therefore, make a motion to the effect that the committee be organized to deal with this matter and report at the next meeting. The motion was seconded.

President Kolmer.—It is now open for discussion and further comments. It is passing over a big burden to the committee to shoulder this enterprise. I believe it is a matter of first rate importance, particularly if the committee can get the cooperation of the society. This is done by the chemical societies with considerable profit to its members. The committee will have a very important task.

Dr. Hartman.—I agree with the chairman that this is an extremely important subject, and getting more and more so. We at least ought to have these procedures listed and I am coming to the opinion that the report ought to include something about the method as to how this procedure should be accomplished. We should have a list of what we approve and let people know of what we approve.

President Kolmer.—I will put the motion now to vote. All in favor say aye; contrary, nay; so ordered.

The American Society of Clinical Pathologists already have in operation a bureau for aiding institutions in securing the services of a pathologist. This can be developed into a department of great service. My special purpose is to ask for an expression of opinion of a bureau for the registration of laboratory technicians; a bureau to which a technician may apply; a bureau to which we may refer. I think this can be developed into a bureau that would be of considerable service. It is now open for any expression of opinion.

Dr. Burdick.—With reference to the machinery already set up, at the suggestion of Dr. Kilduffe some six months ago we started this Service Bureau and at that time I sent a postal card to some nine hundred Class A hospitals of one hundred beds and over, apprising them of this new department. I have about twenty requests from hospitals and possibly as many from clinical pathologists and I have reason to think that we have probably placed one or two clinical pathologists in positions through this bureau. It is a thing which should be brought to your attention and should be developed.

Dr. Sondern.—I move that this matter of registration of technicians be referred to the Executive Committee with power. Motion was seconded.

Dr. Hartman.—The American Society of Bacteriologists has such a bureau and I have tried once or twice to use this and found it quite unsatisfactory. These people are always out of a job and there is a good reason for their being out of a job. If this society takes up this matter they certainly should take measures to know the qualifications of people registering with them.

Dr. Spitz.—May I ask an expression of opinion as to whether the society should derive any monetary benefit from this bureau or is it to be a free service? Of course, if the Executive Committee recommends it, it will be understood that each applicant is competent.

President Kolmer.—Is there further discussion? If not, I will put the question to vote. All in favor that this be referred to the Executive Committee with power say aye; contrary, nay; so ordered.

Dr. Spitz.—I brought up a matter in my paper that is very close to my heart and I believe close to the hearts of other members of the society. Dr. Sondern in his report, mentioned several matters that do not really come under the project of approval of laboratories in the technical sense, but come under the matter of the moral methods of the individual. I might refer specifically to the matter of splitting of fees. I do not see where this would really come under the provision of the standardization. We have in our constitution the clause that the code of ethics of the American Medical Association is our code. I have gone over this code of ethics; I have read it time and again. There are a number of factors in that code that are not applicable to this society. It seems to me that there are many things that should be incorporated in a code of ethics that will cover our relationship, not only with one another, but with the physicians at large, with our technical help and association with hospital connections and also with the public. The time has arrived when this society should have its own code of ethics which can clearly state just exactly what our attitude is on many of these problems. With that thought in view I made the proposal. I now move that you appoint a committee to seriously consider this matter of adopting and endorsing a special code of ethics to cover this society. The motion was seconded.

Dr. Sondern.—I move an amendment to this motion now that the proposition be handed to our committee on revision of by-laws.

Dr. Kilduffe.—I second the amendment.

President Kolmer.—Is there further discussion? It has been moved and seconded that we refer to our Committee on Constitution and By-Laws the matter of drawing up a code of ethics for the American Society of Clinical Pathologists. All in favor say aye; contrary, nay; so ordered.

Dr. Moore.—There was some criticism that our society was not cooperating with the American Medical Association. Dr. Colwell would like to have this society act as the inspectors of laboratories for the standardization of laboratories by the American Medical Association. You know the American Medical Association is composed of units. As the Association has a Hospital Committee which acts on the hospitals that are approved, I would like to have a member of this society in each state if possible become a member of this Hospital Committee; and that member could act with him through the committee in standardizing the laboratories. He feels that every man in this organization is capable of being inspector. He also wishes a minimum standard of laboratories to be drawn up by this organization to be given him to use in standardization of laboratories. We thought it well to make a little closer cooperation, but I think that Dr. Colwell was elected a member of this organization today, so that there will be no trouble in getting cooperation. The next thing we have to do is control entirely the Committee of the American College of Surgeons. There was a point that was brought up yesterday in regard to the free Wassermanns being done by the state. In Illinois we have an Illinois Laboratory Association recently started. The question was brought up at our Tuesday meeting this week how we could stop free Wassermanns from being done by the state where the patients were being charged by the physician for the service. The state was doing it for two purposes: to control the contagion and to give a lower service to the patient. Neither purpose has been fulfilled. I found out that in Illinois, instead of controlling the syphilitic contagion they simply have the state examine the Wassermanns by numbers. I asked the director of the laboratory why they were using this method. He said that they wanted to know how many positive and how many negative Wassermanns there were in the state. I am sure that every laboratory in the state would be very glad to cooperate with the Health Department and send them a report each year or each month of the Wassermanns done in that particular laboratory. A motion was passed that every Wassermann done by the state have the name of the patient and address and that this be sent back to the doctor. We should have a notice sent to the patient that this Wassermann was done at the expense of the state and is public property and that this was done free and there should be no charge on the part of the physician.

President Kolmer.—You brought up three or four subjects. In order to clarify the matter and expedite our consideration, submit them to us in the form of a motion.

Dr. Moore.—I would move that we would cooperate with the secretary of the American Medical Association as closely as we can in his inspection and standardization of laboratories.

Dr. Stillman.—I second the motion.

Dr. Spitz.—That point is already covered by a motion by Dr. Exton that we appoint a committee to confer with the different organizations. I believe the committee already has this function in view.

Dr. Exton.—I think under the circumstances it would be a good plan to put it in the form of a motion and put it to a vote so that they have a better standing when they come to these conventions. The motion is that the committee which is in power to proceed with the matter of standardization be empowered and instructed to confer and cooperate with the authorities of the American Medical Association, the American College of Surgeons and the American Public Health Association with the idea of joining together to better the conditions among laboratories of the country.

President Kolmer.—You have heard the motion, is there any discussion? If not all in favor will please say aye; contrary, nay; so ordered.

Dr. Moore.—I would move then that this organization draw up some form of resolutions to be presented to the state laboratories in regard to the unfair performance of Wassermanns.

President Kolmer.—Is this motion seconded? The motion was seconded.

President Kolmer.—It is now open for discussion.

Dr. Spitz.—I would personally, from a selfish standpoint, like to see the public health laboratories discontinue doing Wassermanns. This is a public health problem. We, of course, quite approve of the various methods being used by the Venereal Disease Boards throughout the country. In my city the public health laboratories are doing Wassermanns and I question whether the evil is sufficient to overcome the good that they are doing. I know there are a lot of patients that are having Wassermanns done by the state that could afford to pay a laboratory fee and there are certain physicians who charge patients for Wassermanns and send them to the state laboratory. I question whether we ought to attempt anything at this time in addition to the other things that we are trying to accomplish which I think are far more important. I believe it would be advisable to let that particular subject alone.

Dr. Graves.—I have a strong feeling that the laboratory, as such, is being crowded out of existence by the public health laboratories. We feel that the performance of Wassermanns is not a public health measure. No ethical laboratory worker would refuse to do a free Wassermann on someone who could not afford to pay for it. We are cutting down the supply of workers by making the field less attractive to them. In my state there has not been a man who has gone into this branch of work for many years. The state is taking over the entire thing. I am ready to offer an amendment that we go on record as being opposed to the State Health and City Health Departments doing Wassermanns on patients other than those coming under their direct control. I would like to offer a suggestion that we appoint one or two counselors in each state to act as a Board of Recourse for this society.

Dr. Lowy.—The free Wassermann is of very little importance; the actual danger to clinical pathologists is that eventually, unless that evil is stopped, they will go very much further than just doing free Wassermanns. Until this organization was formed the clinical pathologists had absolutely no standing. In our city they are giving away hundreds of tons of coal to those who cannot afford to buy it, but if I were to try to get some of this free coal I could not get it; yet they are doing free Wassermanns for people who can well afford to pay for them. The State Board of Health in Rhode Island is attempting to do free blood chemistries. They are infringing upon our right to live. One of our reasons for being in existence is to make a good fair living. We will not be able to do this unless we are willing to stop all this.

Dr. Rhamy.—I think this is the biggest subject that has come before this society. I am from Indiana and our Board of Health not only does every kind of laboratory test, but it advertises what it is doing. The head of the laboratory is not a pathologist at all; he is a health officer and the work is all done by technicians. This man goes to county societies and asks physicians to send their laboratory work to the state. The free Wassermann is the worst of all these. I believe that unless this evil is combated by this society the future of the clinical pathologist will be very dark.

Dr. Stier.—I just want to endorse what the last two speakers have said. In coming through Salt Lake City, I visited the State Laboratory where everything is being done, from tissues on down. It is in charge of a man not fully trained in pathology and not a graduate of medicine. I think it is up to us to take some action to try to curb this state of affairs.

Dr. Owen.—We have the same problem to face in Michigan. I would like to make an amendment that a committee be appointed to take up and investigate this whole question and report to us at some future time.

Dr. Sondern.—I would like to second the amendment of Dr. Owen. This association cannot afford to do anything that is contrary to the interests of public health. I agree with everything that has been said. I would ask the committee that they be most stringent in their investigations.

Dr. Keilty.—I would like to say a word or two on the thought that Dr. Sondern has just raised regarding any action that we may take which may seem to be against public health. These public health societies were organized for the benefit of public health. The

point has been raised several times as to the manner of handling this problem. In Pennsylvania there is a state committee and the head of the laboratory of the department is also a member. It seems to me that if such an organization of committees of the state medical societies were operating through each one of the states they could handle the matter, keep the abuse down, and help along on the good side of it. We ought to be very careful in opposing anything that is for public health.

Dr. Vaughan.—I personally entertain a doubt whether we are justified in taking the stand that because one can afford to pay a fee for a Wassermann one should go to a private laboratory. The man who can afford to pay it pays larger taxes than the man who cannot afford to pay. I think that what should be objected to is the doing of so-called "routine" Wassermanns in our state laboratories. Wherever there is a suspicion of syphilis or outspoken or treated syphilis, I doubt whether we would gain much in the way of cooperation if we try to put any objection up to the health authorities. I cannot see the justice of the practice of many clinicians in sending routine Wassermanns done in the same way as routine hemocytologic or urine examinations to the public health departments.

Dr. Schade.—This question has been thrashed out in Ohio for the last four years. Recently a tissue was sent to the city laboratory in one of the cities in Ohio; the man in charge is a bacteriologist and knows nothing about pathology, so he referred it to another man who had just arrived in the city with very little experience. He made a diagnosis of carcinoma. Fortunately for the patient perhaps, the man sent him to a surgeon. The slides were asked for in this case and were examined and nothing malignant was found. The man said that the slide that showed malignancy was lost. There are a great many evils in this and how to approach them is a question. We, in Ohio, have been on the job for four years; it is a hard proposition. We have taken it up through the local, city, and state societies and we have not arrived anywhere yet.

Dr. Lamb.—From the amount of discussion on the subject something should be done about it. I have had a concrete idea for some time; that is, to point out how much the laboratories are costing the state, then to propose that this service can become self-sustaining and that in order to do so, Wassermanns and tissue examinations should be made only for those who cannot afford to pay for them and that in the case of the Wassermann, for instance, the patient himself sign a statement saying that he is unable to pay the usual fee. The patient could have the service of the state laboratory but he would pay for it. I think there is a thought there that will appeal to the state laboratories. I think that if it is brought before them that there are hundreds and thousands of dollars of possible revenue in the state laboratories they would see the point pretty quickly.

Dr. Lowy.—I would like to bring out another point. That is the absolutely unfair competition. We subscribe to a code of ethics which says that we cannot advertise. The state department goes further than that; it sends out letters paid for by our taxes and competes with us in an unfair way. All that it wants is to do a lot of work so that the director can make a big showing. Now it is perfectly all right for this association not to do anything which would be subversive of public health, but permitting public health authorities to do free work that belongs to clinical pathologists is not fair. We must be treated fairly by our brothers in medicine.

President Kolmer.—I am lost in the maze of amendments. I think I recollect the original motion, I am sure it was seconded, that the matter be referred to a special committee to be appointed by the President to investigate this subject and to report back at our next convention. I am sure that all of you will agree that it is a very important motion. I hope that we will appoint to this committee individuals who will fully realize the importance of the work laid upon them. All in favor say aye; contrary, nay; so ordered.

Dr. Barnett.—I have a matter that I would like to take up. Lately several printed forms of health examinations have come out in some of the medical journals. The laboratory part of the examination was very inadequate. It seems to me that this matter might be referred to the Executive Committee within this organization to take up this matter with the other committees and perhaps make up a minimum requirement for adequate laboratory examinations. I move that a committee in this organization be empowered to take up

tho matter of the inadequate laboratory examination in the health examination with other committees that are making up forms for such examinations. Motion seconded.

President Kolmer.—This motion is now open for discussion. As I understand it we would place upon our Executive Committee the duty during the ensuing year of drawing up what that body would consider a proper series of laboratory examinations to be recommended in conjunction with health examinations. All in favor please say aye, contrary, nay; so ordered. I am ready to receive the report of the nominating committee.

Dr. Schade.—For President for the ensuing year, Dr. Frederick E. Sondern; President-Elect, Dr. William G. Exton; Vice-President, Dr. F. L. Burnett; Secretary-Treasurer, Dr. Ward Burdick; Member of Executive Committee, Dr. Philip Hillkowitz; Member of Board of Censors, Dr. George Ives.

President Kolmer.—You have heard this report, is there any discussion?

Dr. Spitz.—Mr. Chairman, I move that the report be accepted and all of the nominations submitted by the nominating committee be elected. Motion seconded.

President Kolmer.—It has been moved and seconded that this society by a standing vote elect the officers proposed by our nominating committee; all in favor kindly rise. It has been unanimously carried.

The next subject is the selection of the next place of meeting.

Dr. Sondern.—As it has been customary for this association to meet at least near by and approximately at the time of the meeting of the American Medical Association, I would move that the determination of the place and time of the meeting be left to the Executive Committee with power. Motion seconded.

President Kolmer.—You have heard this motion, is there any discussion? If not, all in favor say aye; contrary, nay; so ordered. I believe this terminates the business of the morning. I am not going to be denied the pleasure of ushering the new President into office; that will be reserved for the afternoon session.

SATURDAY, MAY 23, 2 P. M.

The meeting was called to order by President John A. Kolmer and the scientific program was continued. The papers were all read and then discussed as a whole.

The Clinical Significance of Anticomplementary Serum, and Spinal Fluids in the Wassermann Reaction (Kolmer Modification), by Dr. A. H. Sanford, Rochester, Minnesota.

A Standardized Wassermann Report, by Dr A. J. Casselman, Camden, New Jersey.

The Present Status of the Kolmer Complement-Fixation Test for Syphilis as Determined by a Critical Comparison with Numerous Other Methods, by Dr. Robert A. Kilduffe, Atlantic City, New Jersey.

Comparison of Results with Kolmer Wassermann Method and Kahn Precipitation Test, by Dr. Robert G. Owen, Detroit, Michigan.

Kahn's Precipitation Reaction as Compared to Kolmer's Complement-Fixation Test, by Dr. A. S. Giordano, South Bend, Indiana

A Clinical Study of the Kolmer and Kahn Reactions in Syphilis, by Dr. R. L. Kelly, Louisville, Kentucky. (By invitation)

The Meinicke Reaction as Compared with the Wassermann in One Thousand Specimens of Blood Sera, by Dr. A. M P Saunders, Dunning, Illinois. Paper read by Dr. Herman Spitz.

President John A. Kolmer with a few well chosen words then ushered Dr. Frederick E. Sondern into office.

Dr. Sondern.—I appreciate fully this honor I am grateful to you for your confidence. I will do my best to merit what you have done.

You have put before your officers and representatives two very difficult tasks. The more difficult of the two is this consideration of what is proper for Public Health, State, County and City Laboratories to do in the way of work and what is not proper. This is one of the most difficult problems I know of, but I am going to try, with the help of the committee, to solve it as best we can. I do not think we can solve it entirely, but we are going to try to bring you our best explanation of it. I am serious in this matter and I want to assure you that your officers are going to do all they can to carry out your wishes

in this long interval.   I thank you again for this honor and as I said before, I am going to do my very best.

The scientific program was continued and the papers that had been read were opened for discussion.   Discussed by Dr. Martin, Dr. A. J. Casselman, Dr. Henry Stewart, Dr. Herman Spitz, Dr. H. J. Nichols, Dr. Robert A. Keilty, Dr. Frank W. Hartman, Dr. R. A. Kilduffe, Dr. John A. Kolmer.

There being some time left, Dr. B. S. Parks read his paper, The Bactericidal Action of Whole Blood as Determined by the Heist-Lacy Method.   (By invitation.)

The meeting was then adjourned.

EDITORIALS

The Philadelphia Meeting

IN a perusal of the transactions of the American Society of Clinical Pathologists which appear elsewhere in this issue, one will gain an excellent insight into the character of the agenda. While the purposes of the society are known to any who care to read the constitution and by-laws or the brochures which have been published by the society, the real test of any organization's value is in its accomplishments in and out of business session.

During its relatively short existence the A. S. C. P. has already to its credit: the evolution of an idea; the gathering to its standard of representative men and leaders in clinical pathologic thought; the acquiring of full recognition from older medical organizations; and the inception of appropriate steps towards the realization of the idea,—the ideals for which the society was organized.

We need not discuss here the high caliber of the scientific contributions, since they will appear later on their own merits, but the journal takes real pleasure at this time in congratulating the society on its accomplishments, and on its successful annual convention.

—W. T. V.

Cholesterolemia and the Complement-Fixation Test for Syphilis

DESPITE the enormous amount of study which has been devoted to the complement-fixation test in syphilis we are still without definite knowledge as to the exact mechanism responsible for the production of a positive reaction.

"While lipoidal extracts, as well as normal and luetic serums, may separately absorb or fix a small amount of complement, a mixture of a suitable extract and a suitable (syphilitic) serum is capable of fixing large amounts of complement"[1]—and that is all that is known, so far, of the mechanism concerned in the production of the reaction in syphilis.

It is highly probable that the true explanation of the reaction may lie in the field of colloid chemistry and that changes in the surface tension and alterations in the character and dispersion of colloidal particles in the serum, complement, and antigen are factors of decided importance.

That lipoids are of extreme importance was first brought out by the introduction of cholesterolized antigens, concerning the reliability and specificity of which there has been much discussion. From the fact that the addition of cholesterol to an antigen led to an increase in the number of positive reactions obtained with it, to a speculation as to the effects of an increase of cholesterol in the fluids examined was a natural step.

Among the early observers to credit an increased cholesterol content as a factor capable of producing a positive reaction was Pighini[2] who, finding a large amount of cholesterol in 88 per cent of Wassermann positive spinal fluids, concluded that the cholesterol content was an important factor, if not the main cause, of the positive reactions.

Henes[3] not only believes that the interpretation of the significance of a positive reaction must be directly governed by the cholesterol content of the specimen—of less significance when the cholesterol is high than when it is low or diminished—but also maintains that a high cholesterol content may be the direct cause of a positive reaction.

This assertion, if true, would be of vital importance to both serologist and clinician as depriving the complement-fixation test in syphilis of all significance unless a cholesterol determination is simultaneously performed, and unless the specimen examined showed a normal or diminished amount.

The statement, however, has not been allowed to pass unchallenged.

Cholesterol forms a part of every mixed diet and, while the power of the body to synthesize cholesterol from cholesterol-free substances is still a matter of doubt, that the blood cholesterol content can be deliberately increased by the ingestion of foodstuffs rich in this substance has been definitely shown.

If, therefore, a mere increase in the blood cholesterol is a factor of im-

portance in the production of a positive complement-fixation test, it should be easily demonstrable by artificially producing a hypercholesterolemia by feeding experiments and sera so obtained should exhibit a positive complement-fixation test.

Such a series of experiments has been reported by Craig and Williams.[4]

These investigators chose for their experiments the rabbit because, first, this animal is known to give a positive complement-fixation reaction after experimental infection with the Spirocheta pallida, and, second, because it has been demonstrated that the blood cholesterol of these animals can be definitely increased by feeding cholesterol.

To a series of ten rabbits known to give consistently negative complement-fixation reactions (over a period of ten days preceding the experiment), cholesterol was fed in large amounts.

Prior to the experiment cholesterol values were shown to range from 85.5 to 133 milligrams per 100 centimeters of blood. Similar determinations during and after the termination of the feeding experiments gave values showing an increase of from 284.3 per cent to 680 per cent above normal values. Complement-fixation tests during these periods of extreme hypercholesterolemia were consistently negative.

It may be thought that such experiments, while of interest, are only hypothetically comparable to similar conditions in the human being. It is permissible, therefore, to speculate as to what would have been the results under similar conditions of hypercholesterolemia in human serum and the query finds an answer in the work of Thalhimer and Hogan.[5]

The plan pursued by these investigators was to make a quantitative complement-fixation test after the method described by Kolmer and thus determine with great exactitude the complement fixing value and then, after adding to the positive serum a known negative serum having a high cholesterol content, to repeat the test on the assumption that, if cholesterol affects the results of complement-fixation tests, then increasing the cholesterol content of the tested serum should also increase the strength of the reaction.

The hypercholesterolemic sera were obtained from cases of pregnancy, jaundice, diabetes, and nephritis and the cholesterol content ranged from 250 milligrams per cent to 833 milligrams per 100 centimeters of serum, the normal value in the human being ranging from 160 to 180 milligrams per cent.

The negative sera which, it is to be noted, were negative in spite of their high cholesterol content, were all tested for the presence of natural antisheep hemolysins which, if present, were removed by absorption.

The results of the experiment were entirely uniform: The hypercholesterolemic sera were negative in the first place and when added to known positive sera in a quantitative test not only failed to increase the strength of the reaction but this on the contrary, was decreased owing to the dilution of the positive sera by the addition of the negative sera. These observers, therefore, concluded that hypercholesterolemia was not a factor in the production of a positive complement-fixation reaction in syphilis and cannot thus be the cause of false or nonspecific positive reactions.

The pathologic factors concerned in the production of hypercholesterolemia are not well understood. While it is true that some of the methods recognized as at times giving false positive reactions with a majority of the methods in common use, such as diabetes and pregnancy, are not infrequently associated with hypercholesterolemia—though this is not constant—it is equally true that in other conditions, such as pneumonia during the febrile stage in which false positive reactions are not infrequently obtained, the blood cholesterol is markedly decreased.

Syphilis, however, is the one disease in which positive complement-fixation reactions are consistently found. If hypercholesterolemia is responsible even in part for the production of the positive reaction, then hypercholesterolemia should be consistently associated with syphilis.

An investigation along these lines is reported by McFarland[6] who found that of a series of serologically positive syphilitics only 6.8 per cent showed a high cholesterol value, 75.9 per cent having a "medium" content, and 17.2 per cent having low values. He concludes, therefore, that there is no relation between the cholesterol content of the serum and the occurrence of a positive reaction.

Of interest, also, is the work of Levinson, Landenberger, and Howell[7] who determined the cholesterol content of 53 positive spinal fluids, examining 25 by both qualitative and quantitative methods, and 28 by qualitative methods alone. In none could a reading be made by the Bloor method nor was there any inhibition of hemolysis by the saponin test.

In only three fluids—one luetic and meningitic combined, and two from general paresis—was it possible to demonstrate the presence of cholesterol.

It appears fair to conclude, therefore, that the presence of cholesterol has no bearing upon the production of positive complement-fixation reactions in luetic spinal fluids, and that pathologic or experimental increase of cholesterol in either the blood or spinal fluid has no influence upon the production of a positive complement-fixation reaction in syphilis and no bearing upon the interpretation of the reaction.

REFERENCES

[1]Kolmer, J. A.: Infection, Immunity, and Specific Therapy, Philadelphia, ed. 2, 1920, W. B. Saunders and Co., p. 429.

[2]Pighini: Ueber den cholest. der lumbalhussigkeit ein Gusteskrankeiten, Hoppe-Seyler's Ztschr. f. physiol. Chem., 1909, lxi, 508. Cited by Levinson, Landenberger, and Howell, Note 7.

[3]Henes, E.: Cholesterolemia and the Wassermann Reaction, Jour. Am. Med. Assn., 1915, lxiv, 1969; ibid, Am. Jour. Syph., 1920, iv, 1.

[4]Craig, C. F., and Williams, W. C.: Experimental Observations upon the Effect of Cholesterolemia on the Results of the Wassermann Test, Am. Jour. Syph., July, 1921, v, 3.

[5]Thalhimer, W., and Hogan, B. M.: The Inability of Serum with a High Cholesterol Content to Increase the Strength of the Wassermann Reaction, Arch. f. Dermat. u. Syph., April, 1923, vii, 4.

[6]McFarland, A. R.: Studies in the Blood Cholesterol in Syphilis, Arch. f. Dermat. u. Syph., July, 1922, vi, 39.

[7]Levinson, A., Landenberger, A., and Howell, K.: Cholesterol in the Cerebrospinal Fluid, Am. Jour. Syph., April, 1921, clxi, 589.

—R. A. K.

Color Scales and Complement-Fixation Reactions

THE occurrence of a positive complement-fixation reaction is evidenced and its degree determined by the absence of hemolysis in the corpuscle suspension added as an indicator.

Because of the quantitatively adjusted proportions of the complement, antigen, and serum doses on the one hand, and the amboceptor and corpuscle doses on the other, it is obvious that various readings are possible from complete hemolysis to its complete inhibition, and to record these gradations the so-called Citron scale has long been in use and is familiar to everyone.

As concerned with the development of uniformity in the performance and reading of the test, attention has been again directed toward refinements in the use of the Citron scale as a means of determining with greater or less exactitude the intensity of the reactions obtained.

In a recent communication, Gilbert, Kelly, and Moore[1] report the result of their studies in this direction culminating in the construction of a new and carefully prepared indicator standard or color scale which they suggest as tending toward uniformity in reporting the results of the test.

From time to time in the literature of the complement-fixation test as applied to the diagnosis of syphilis, various methods of preparing the corpuscle suspension have been proposed, from attempts to standardize the suspension by actually counting the cells, to attempts at standardization by the estimation of the hemoglobin; none, however, has attained any great vogue nor, in the last analysis, are they a necessary or even a logical concomitant of a "standard" technic, nor do they achieve more than an apparent exactitude.

One of the underlying purposes of attempts to develop a "standard" technic is to render possible the comparison of the results obtained by different workers on the same or different sera or of the same worker on serum obtained from the same individual at different times.

That this can be brought about with entire satisfaction by attempts to standardize the corpuscle suspension is open to question.

In the first place, while it is quite possible that the individual worker may more closely approximate his own results from day to day by the use of a color scale, it by no means follows that his results may be quantitatively compared with those of other workers, even though they are using the same scale, unless they are using an identical technic in the performance of the test.

Inasmuch as the occurrence of hemolysis or its inhibition is directly related to and influenced by the quantitative relations existing between the various reagents, no exactitude in the preparation of reading scales will render possible the exact comparison of results by two methods in which the other quantitative factors are markedly at variance. The sensitivity of the antigen; the period and temperature of the primary incubation; the serum and complement dose must all be considered and unless all are comparable, the tests may only be compared in a gross way as to whether they are negative or positive—

and even here, there may be a perceptible or even pronounced disparity when one technic is much more delicate than another.

Reading scales may well serve to achieve a little more exact differentiation between a plus-four and a plus-three in the same laboratory with the same technic but cannot per se serve to impart a strictly quantitative factor, for methods utilizing a single or even a double dose of serum are essentially qualitative in character and serve merely to establish the presence or absence of reacting substances.

That it is important to have some means of estimating the strength of reactions is admitted and there can be little debate as to the necessity of a quantitative factor in any "standard" technic; but as long as we remain uninformed as to the underlying mechanism of the complement-fixation reaction; as long as we are ignorant as to the substances producing the positive reaction, just so long will attempts to measure the strength of the reaction be clouded with difficulty and more or less empirical.

There is, it is true, a difference in the intensity of a plus-four and a plus-one reaction read with or without a scale, but the difference is quantitative only in the sense of a quantitative difference between a "large amount" and a "trace" of albumin in a qualitative test in the urine; a gross difference—no more and no less.

Serologists and clinicians are coming to accord as to the necessity for a quantitative factor; the question is concerning the best method whereby it may be obtained.

In the present state of our knowledge, as has been discussed elsewhere,[2] a quantitative factor appears possible in only one of three ways:

1. By determining how great a number of antigen units a given quantity of tested serum can utilize in the presence of a set dose of complement.

2. By determining how great a number of complement units a given quantity of serum can fix in the presence of a set dose of antigen.

3. By determining how small an amount of serum is capable of producing any degree of fixation, however minor, in the presence of a set dose of complement and antigen.

Of these the last has the most advantages and the least disadvantages and is altogether the most feasible.

It is apparent that a serum which is capable of giving fixation of plus-two degree in an amount of 0.0025 c.c. contains more reagin than one which gives fixation only in 0.05 c.c. even though the degree of fixation be plus-four in the latter amount. If emphasis is placed—as it may be—not upon the *degree* of fixation but upon the *quantity of serum* with which it is obtained, the reaction becomes more strictly quantitative and, where different workers are using even varying technics but all are using graded doses of serum, their results become comparable in degree if the same or comparable doses of serum are tested, even though the plus-three of one worker be the plus-four of another.

If both obtain fixation with 0.0025 c.c. of tested serum both have obtained a reaction the strength of which may be, at least, compared; though refinements of technic may influence appreciably the degree of inhibition of hemolysis or even its occurrence in a stated serum dose.

It would appear that uniformity of results as related to the strength of the reaction obtained is more likely to be attained through the use of graded amounts of tested serum which may be more easily prepared than reading scales, if not, perhaps, more accurately, and which present, moreover, a logical as well as a ready means of measuring that upon which the strength of the reaction depends—the reagin content of the serum.

REFERENCES

[1]Gilbert R. Kelly, M. F., and Moore, A. C.: The Reading of Complement-Fixation Tests by the Citron Scale as Compared with a Method Using a Color Scale, JOUR. LAB. AND CLIN. MED., April, 1925, x, 552.
[2]Kilduffe, R. A.: Concerning the Necessity for a Quantitative Factor in a Standard Complement-Fixation Test for Syphilis and the Methods Whereby it may be Obtained, Ann. Clin. Med., January, 1925, iii, 481.

—R. A. K.

Notice

The continually increasing cost of production in the printing business makes it necessary that we raise the subscription price of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

Beginning with the October issue, the subscription price will be increased $1.50 per year. The size of the Journal will be increased possibly two hundred pages during the year. This increase is necessary in order that we can accommodate the large amount of worth-while material that is being continually offered to the Journal and that should be published.

BOOK REVIEWS

Concealed Tuberculosis*

THE author takes the stand that "that tired feeling" in individuals in whom no physical cause can be found and which is usually attributed to neurasthenia, is often due to latent active tuberculosis. In detecting the source of the infection, he employs the subcutaneous tuberculin test using as his criterion a febrile reaction. He requires no focal reaction since he maintains that often in these cases the infection is so well concealed that no focal reaction can be recognized by present methods of examination. The cutaneous tuberculin reaction is not as trustworthy. Pathologic proof of tuberculous infection is not easy because these patients rarely die within the period of observation.

The treatment recommended is the usual "preventorium" treatment.

The book is essentially a brief for the recognition of latent active tuberculosis without localizing foci as a cause for various neurasthenic symptoms grouped under the popular term "The Tired Sickness," and for the more general use of the subcutaneous tuberculin test in the recognition of these cases.

Principles of Biochemistry†

THE second edition of Robertson's *Principles of Biochemistry* contains the usual proportion of alterations and additions, necessitated by advances in our knowledge of the subject. The sections dealing with the regulation of the neutrality of the blood and with metabolism in diabetes have been considerably expanded. The chapter on growth has been greatly altered and a new chapter has been added on the relation of growth to diet. New sections deal with the racemized proteins, the functions of the nucleic acids and on the environment as a factor in the evolution of the higher animals.

After considering the inorganic foodstuffs and the significance of foodstuffs in general, the author discusses the chemistry of the carbohydrates, the hydroaromatic derivatives, fats, proteins and amino acids, nucleic acids and the nitrogenous bases. He next takes up the hydrolyzing enzymes and finally the digestion and assimilation of various foodstuffs.

The second portion of the book deals with the properties of protoplasm. This is chiefly a consideration of the colloidal chemistry of the body.

Part Three is of unusual interest in its mode of presentation. In it the author deals with the chemical correlation of the tissues. First, he discusses from the biochemical viewpoint the vehicles of correlation, the blood and lymph. Second, he describes the chemical correlation of respiratory activity, the chemical regulation of the circulatory system, the chemical correlation of the processes of digestion, of the organs of generation and the chemical regulation of metabolism.

Part Four deals with the chemical processes which underlie and accompany life's phenomena. This includes the intermediate metabolism of the various foodstuffs, the energy transformations in living organisms, fertilization, early development, growth and the relation of growth to diet, memory and sleep.

Section Five deals with the waste products and Section Six with the animal body as a machine, or the energy balance of the organism.

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*Serum Diagnosis of Syphilis by Precipitation**

THE great interest which has developed within the last two years in the various precipitation tests for syphilis, particularly the Kahn test, has rendered it highly desirable that an authoritative discussion of the reaction in all of its phases be presented in one volume.

The author presents, in all necessary detail, historical notes, the governing principles of precipitation with their application, and a detailed description of his test as applied in practice.

Although there is distinct difference of opinion regarding the ultimate reliability of precipitation tests as opposed to the standard Wassermann reaction, the fact that according to the author's figures, his reaction as now employed, agrees with the standard technic in 97 per cent of tests, demonstrates that it has a distinct place in the serology of syphilis. In many localities indeed, it is being relied upon almost entirely as a diagnostic procedure, and a clear understanding of its mechanism, as brought out in this book is therefore highly desirable.

The argument for the test would perhaps have been slightly more complete had the author taken up in a special section, a discussion of those criticisms that have appeared in the literature.

Compend of Genito-Urinary Diseases and Syphilis†

AS a compend, this is very good. All genitourinary conditions except perhaps the exceedingly rare, are briefly discussed and the more generally accepted views are presented. Recently developed procedures and methods of treatment such as tryparsamide in neurosyphilis, mercurochrome, etc., are incorporated. Good illustrations are included.

*Serum Diagnosis of Syphilis by Precipitation. Governing Principles, Procedure, and Clinical Application of the Kahn Precipitation Test. By. R. L. Kahn, M.S., D.Sc. Michigan Dept. of Health. Pp. 237. Cloth. Price $3.00. Williams & Wilkins Co., Baltimore, 1925.

†A Compend of Genito-Urinary Diseases and Syphilis. By Charles S. Hirsch, M.D. Fourth edition. revised. 44 illustrations. Pp. 337. Cloth. Price $2.00 P. Blakiston's Son & Co., Philadelphia, 1925.

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CLINICAL AND EXPERIMENTAL

THE PULPLESS TOOTH FROM A BACTERIOLOGIC AND EXPERIMENTAL STANDPOINT*

By Russell L. Haden, M.D., Kansas City, Mo.

THE thoughtful clinician in evaluating the pulpless tooth as a factor in disease asks three questions: 1. How frequently is the pulpless tooth infected; how far can one translate radiographic evidence of infection into terms of bacteria? 2. Are the bacteria found in areas of dental infection able to produce disease; if so, with what frequency? 3. In individual cases what experimental proof is there that a focus of dental infection bears a causal relation to the patient's symptoms? The answers to these questions must constitute the scientific basis for determining the rôle that dental infection plays in the causation of chronic infectious disease. The problem is largely a bacteriologic one, yet it is surprising how little data is available concerning the points mentioned. There is not in the literature a single comprehensive bacteriologic study of dental infection by modern methods; relatively little work has been done to determine the pathogenicity of mouth streptococci by the correct technic; very little experimental proof concerning the causal relation of a focus to a disease in individual cases is available except that of Rosenow and coworkers and of Price.[6] I have attempted by cultures of dental infection and by certain animal experiments to obtain further facts in answer to the questions asked. This data is presented below.

1. THE BACTERIOLOGIC STATUS OF THE PULPLESS TOOTH

To determine, if possible, the exact bacteriologic status of the pulpless tooth, we have cultured the apices and periapical tissues of a large number of pulpless teeth. A quantitative technic has been employed by which the

number of bacteria present in the tissues cultured can be determined. The extractions have all been done by Charles W. Keeling and Carl D. Lucas, whose cordial cooperation has made possible this study. The technic used in obtaining the culture material is as follows: The teeth are first thoroughly scrubbed off with gauze, and the gums are then painted with tincture of iodine followed by alcohol. The field of operation is packed off with sterile gauze and the tooth extracted with sterile forceps. The apex of the tooth is cut off with sterile forceps directly into a sterile tube containing about 1 c.c. of salt solution and a small amount of sand.

A.   B.

Fig. 1.—Cultures from periapical dental infection in deep tubes of glucose brain broth agar. A, culture in which the growth is uniform throughout the tube; B, culture in which the organism grows only about three-fourths of the way to the top of the tube.

In making the bacteriologic cultures we have followed closely the technic of Rosenow. Each tooth has been cultured in deep tubes of glucose brain broth and glucose brain agar. These mediums afford all degrees of oxygen tension and are thus especially favorable for the growth of the streptococci found in chronic focal lesions. The technic used in the preparations of the mediums has been described in detail elsewhere.[1] The tube containing the root tip in the sterile sand and salt solution is well shaken to macerate the tissue on the tip of the tooth as completely as possible. The mediums are inoculated by pouring the salt solution containing the suspended tissue into a deep tube of glucose

brain agar which has been heated and allowed to cool to 40° C. The small amount of salt solution remaining in the tube is then poured into a tube of glucose brain broth. The inoculated tubes are incubated at 37° C. for twenty-four to forty-eight hours. The agar tube is employed only to determine the number of bacteria present. The appearance of such a positive culture is shown in Fig. 1. Smears are made from the broth tube to determine the type of organism and the broth culture is then used for animal inoculation.

In the statistics to be presented, I have included cultures of the incisors, cuspids and bicuspids only, since extraction of the molars without mouth contamination is often quite difficult. The different steps in the technic have been repeatedly checked bacteriologically to detect possible errors. As a further check on technic, I have constantly cultured vital teeth to determine the percentage of error.

TABLE I

RESULTS OF QUANTITATIVE CULTURES FROM PERIAPICAL DENTAL INFECTIONS

GROUP	NUMBER CULTURED	NUMBER SHOWING IN DEEP AGAR TUBE			NUMBER STERILE IN BROTH
		1 OR MORE COLONIES	10 OR MORE COLONIES	OVER 100 COLONIES	
Vital teeth	392	14%	5%	1%	46%
Pulpless teeth with negative radiograph	490	54%	44%	24%	18%
Pulpless teeth with positive radiograph	425	70%	63%	44%	9%
All pulpless teeth	915	61%	51%	33%	14%
Total	1307				

The results of the cultures made in the manner outlined of 1307 teeth are shown in Table I. I have not included in the series any teeth in which there was a question of contamination from saliva, lips, tongue or otherwise at the time of extraction. Likewise, I have excluded teeth showing pyorrhea. The teeth cultured are fairly equally divided between vital teeth, pulpless teeth with negative radiograph, and pulpless teeth with positive radiograph. Three hundred ninety-two vital teeth have been cultured. Of these 14 per cent have shown 1 or more colonies in a deep agar tube, 5 per cent 10 or more colonies and 1 per cent have had over 100 colonies. It seems reasonable to take 10 or more colonies as an arbitrary number as indicating sufficient infection to be of possible importance from a systemic standpoint. These results indicate also the percentage of error which must be allowed for interpreting the results in pulpless teeth. The positive cultures do not all represent, however, errors in technic. Some of the teeth considered as vital were probably pulpless, since all were not tested for vitality, and others had large cavities and might well have had infected pulps. Forty-six per cent of the broth cultures from these teeth were sterile.

Four hundred twenty-five pulpless teeth with positive radiograph are included. Of these 70 per cent showed 1 or more colonies in the deep agar tube, 63 per cent 10 or more colonies, and 44 per cent over 100 colonies in the deep tube. Nine per cent of the broth cultures in this group were sterile.

The significant findings here are the facts that 40 per cent of the teeth with positive radiographic evidence of infection showed less than 10 colonies and 9 per cent were sterile in broth. It is evident that in many cases the infection had run its course and healing had taken place so far as the presence of bacteria is concerned.

Four hundred ninety pulpless teeth with negative radiograph have been cultured. Fifty-four per cent of these have had 1 or more colonies in the deep agar tube, 44 per cent 10 or more colonies, and 24 per cent over 100 colonies; 18 per cent were sterile in broth. I realize that there may be much discussion and difference of opinion concerning what radiographic criteria should be employed in placing teeth in this group. We have taken a conservative attitude as to what is positive or negative. The striking thing here is the high percentage of positive cultures. The incidence of infection is almost as high as in those with a positive radiograph. Infection in the tooth with a negative radiograph is probably more serious from the systemic standpoint that when the radiograph is positive, since little resistance on the part of the body is indicated, and absorption is probably more rapid. The organisms recovered have been streptococci in pure culture or mixed with staphylococci. Only rarely have other organisms been encountered.

I should like to emphasize that these cultures so made, show only the bacteriologic status at the time the culture is taken. It tells nothing as to what was there yesterday, or would have been present thereafter. It must also be granted that some of the negative cultures might have been positive if cultured in other ways, although the mediums used are the most favorable known for the growth of nonhemolytic streptococci. Several times during the past two years I have compiled statistics concerning the cultures and the percentage has remained almost constant.

These results show that infection is actually present around the root tips, tells something about the frequency of the infection, and gives an idea of the number of bacteria present. They also show that radiographic findings, while of aid, cannot be translated into terms of bacteria, nor can infection around the tip of a pulpless tooth be ruled out on a radiographic basis.

2. THE PATHOGENICITY OF THE BACTERIA FROM DENTAL FOCI

To determine the disease-producing power of the bacteria from dental foci we have injected rabbits with the original broth cultures recovered from the root tip. Two rabbits have been injected routinely with 5 c.c. each of the culture from a single tooth or with the mixed cultures from several teeth. In most instances the animals do not die as a result of the injection. If they do not, they are killed at intervals of three to six days after the injection, and the various organs examined carefully for lesions.

Other workers of course have determined the pathogenicity of mouth organisms by the injection of cultures into animals. Such a study is that of Hartzell and Henrici.[2] However, most workers have not used, for the injection, organisms grown under partial oxygen tension, or made the injections soon after isolation. These points are vitally necessary if one is to determine the true pathogenicity of the organisms. Disease-producing power in the

group dealt with here is quickly lost when these fundamental requirements are not met.

We have been able to reproduce in rabbits almost all types of lesions which can be caused by the intravenous dissemination of bacteria. The frequency with which the main groups of lesions occur is shown in Table II. The lesion most commonly found is joint involvement, the second kidney lesions, the third muscle, followed by a smaller number in endocardium, myocardium, brain, eye, and stomach and duodeum. The incidence of involvement in the animal corresponds closely to the incidence of lesions due to chronic foci, as observed clinically in man. In addition to the lesions enumerated many others have been observed such as tenosynovitis, cholecystitis, enteritis, bladder involvement, nerve lesions, and so forth.

TABLE II

LOCALIZATION OF BACTERIA ISOLATED FROM DENTAL INFECTION
(INTRAVENOUS INJECTION IN RABBITS)

NUMBER OF ANIMALS INJECTED	NUMBER OF PATIENTS	PERCENTAGE OF ANIMALS SHOWING LESIONS IN							
		JOINT	KIDNEY	MUSCLE	ENDO-CARDIUM	MYO-CARDIUM	BRAIN	EYE	STOMACH AND DUO-DENUM
761	255	58	34	24	18	11	6	18	13

The high incidence of lesions shows clearly the great disease-producing power of the bacteria from chronic foci and leaves no doubt that such organisms are capable of causing disease in man to a high degree.

3. EXPERIMENTAL PROOF IN INDIVIDUAL CASES OF A CAUSAL RELATION
OF DENTAL INFECTION TO SYSTEMIC DISEASE

The data so far presented, however, gives in individual cases no proof of a causal relation of the dental infection to the systemic disease. Certainly the most convincing proof we have of the relation of a focus to a disease is the reproduction in animals of the condition from which the patient suffers with the organism recovered from the patient's focus. Such proof concerns the theory of Rosenow that bacteria tend to localize in certain tissues of the body due to some peculiar inherent property. The truth of the theory has been conclusively demonstrated by Rosenow and his coworkers. I have been able to present confirmatory results in diseases of the eye,[1] of the stomach,[3] of the kidney,[4] and in cases of onychia.[5] The proof seems absolute that bacteria do have such a selective tendency. Those who have questioned the theory after experimentation have failed to observe the necessary requirements as far as oxygen tension and rapidity of work is concerned. The demonstration then that animals injected with the bacteria develop lesions similar to those of the patient is the strongest evidence as to causal relation; and, likewise, evidence that in the patient and in the animal, we are dealing with the same organism. The proof that in certain cases there is this causal relation of dental infection to systemic disease is best presented by a few case histories with the protocols of animal inoculations, with the organisms recovered from chronic dental foci in the patient.

CASE REPORTS

Endocarditis and Auricular Fibrillation

CASE 1.—*History.*—L. C. H., widow, a clerk, age sixty, complained of heart trouble. She had had chorea first at age of twelve with recurrent attacks for several years. At fourteen, she had had diphtheria and at twenty-three scarlet fever. Eight years before she had scleritis. For several years she had albumin and pus in the urine. At one time removal of kidney was considered on account of the pyuria.

The patient stated she had been well up to 1912, eleven years before admission, when she had a severe attack of influenza. Two weeks later she began to have arthritis which persisted for six months. She was then well for several months, after which she began to have attacks of rapid and irregular heart. She had to give up work for seven weeks at this time on account of the heart symptoms. About once a year since this initial attack she had had an attack of heart trouble incapacitating her for work for six weeks to four months. During the past year the attacks have been occurring every few days and lasting only a few days at a time. The symptoms were worse on exertion. At times the ankles were swollen. Recently the patient had been to the Mayo Clinic where a diagnosis of paroxysmal auricular fibrillation was made.

On examination on admission, there was a definite aortic insufficiency without demonstrable cardiac enlargement. The heart rate was slow and regular except for an occa-

Fig. 2.—Large vegetations on valves of heart of rabbit injected with the culture from infected teeth of Patient 1, who was suffering from auricular fibrillation and aortic insufficiency.

sional extra systole. The blood pressure was 140/70. The urine showed a few pus cells in clumps.

There were eleven pulpless teeth, only four of which showed definite radiographic evidence of infection.

Animal Inoculations.—The lower right second bicuspid and first and second molars were extracted first. All showed a profuse growth of nonhemolytic streptococci. Two rabbits were injected. One had at autopsy a few endocardial vegetations, a few abscesses in the medulla of the kidney, and a small amount of purulent fluid in the joint. The other rabbit showed a massive vegetative endocarditis of the tricuspid valve (Fig. 2), a few lesions in the myocardium, and slight involvement of the joints. One rabbit was injected with the cultures from the lower left bicuspid and second molar. At autopsy a few vegetations on the heart valves, numerous small abscesses in the wall of the left ventricle, a purulent arthritis and a few kidney abscesses were found. Two rabbits were injected with the cultures from the remaining teeth. One was dead the following day. There were many hemorrhages in the endocardium of the left ventricle, and at the base of the papillary muscles. There were also a few hemorrhages and small vegetations in the right auricle near the ventricle.

The other rabbit died two days after injection and at autopsy showed only early vegetations on the mitral and the tricuspid valves, and mural thrombi in the right auricle.

Chronic Arthritis

CASE 2.—*History.*—R. K., A salesman, age twenty-seven, complained of rheumatism. He had never been sick until the present illness. The tonsils had been removed in 1919, four years before. The present illness had begun four weeks before admission with an eruption on the hands and trunk followed by an arthritis of the right wrist. When first seen all the larger joints and the finger joints were red, painful, and stiff. He could walk only with the aid of crutches. His eyes had been injected and painful. There was a raised pink macular eruption with clearing centers over the shoulders, arms, chest, and back, resembling erythema multiforme. The leucocytes were 13,350. The urine was negative and the Wassermann test negative.

There were only two pulpless teeth both of which showed bone absorption at the apex (Fig. 3). These were extracted. Both showed a profuse growth of green producing streptococci.

The patient had come in on crutches. He felt better immediately after the teeth were extracted. The following day all the joint symptoms had disappeared. Two days later he again came in on crutches. Examination showed that the sockets were not draining. They were opened up again. The symptoms disappeared immediately and did not return. The skin eruption also cleared up quickly.

Animal Inoculations.—Two rabbits were injected. Both at autopsy had a very marked purulent arthritis and kidney abscesses. The organism injected was recovered in pure culture from the joint and kidney lesions.

Fig. 3.—Infected teeth of Patient 2. The cultures from these teeth produced very marked arthritis in rabbits on intravenous injection.

Acute Facial Paralysis

CASE 3.—*History.*—L. M. H., a plumber, age thirty-nine, complained of facial paralysis. One year previously, he had suffered from dizzy spells and on examination was found to have albumin in the urine. The facial paralysis had appeared suddenly five days ago without any pain.

On examination the right side of the face was completely paralyzed. No other cranial nerves were involved. The blood pressure was 130/85. There was no anemia. The Wassermann test was negative. The urine examination showed a specific gravity of 1.023, and albumin four-plus, with many granular casts. The phenolsulphonephthalein excretion and the blood urea nitrogen were within normal limits. The dental radiographs showed four pulpless teeth all presenting evidence of periapical infection.

Animal Inoculations.—Three rabbits were injected with the mixed cultures from the four extracted teeth. One developed a paralysis of the hind legs forty-eight hours after injection and was killed. At autopsy all organs were negative except the brain and spinal cord which showed marked injection, some exudate at the base of the brain (Fig. 4) and hemorrhage in the caudal end of the cord. The second rabbit showed no symptoms at any time and at autopsy only some vegetations on the heart valves were found. The third rabbit, two days after injection, had a paralysis of the left ear and a pericorneal injection which gradually cleared up. When killed the only lesion found was a purulent arthritis.

Recurrent Hyalitis

CASE 4.—*History.*—L. P., a steam fitter, age twenty-five, was first seen June 23, 1926. He complained of something flying around in front of the right eye. This was first noticed

about three weeks previously following an attack of influenza, and had become gradually worse.

The fundus was seen poorly. No hemorrhages were present. Many fine dust-like opacities and some larger ones were floating freely in the vitreous, also shreds of hyaloid tissue to which were attached numerous dust-like opacities. There was no opacity of any kind to be found in the aqueous or on the back of the cornea and no inflammation was apparent. Vision in right eye 20/100.

Under treatment the vision became better, 20/50, and at one time 20/40. Between June, 1920, and February, 1922, the patient has had four light attacks and one very severe one, which left the eye almost without a reflex. These exacerbations did not come on suddenly, as they would have if they had been recurrent hemorrhages. From the time of his first visit the patient was urged to have all his pulpless teeth extracted, but several were not removed.

A general physical examination February 24, 1922, was negative. The tonsils had been removed. Blood: red cells, 4,632,000; hemoglobin, 90 per cent; white cells, 7,400; differentia count; PMN, 55.5 per cent; PME, 4.5 per cent; PMB, 2.0 per cent; SM, 25.5 per cent; LM and Trans., 12.5 per cent. The urine showed no abnormality and the Was-

A.   B.
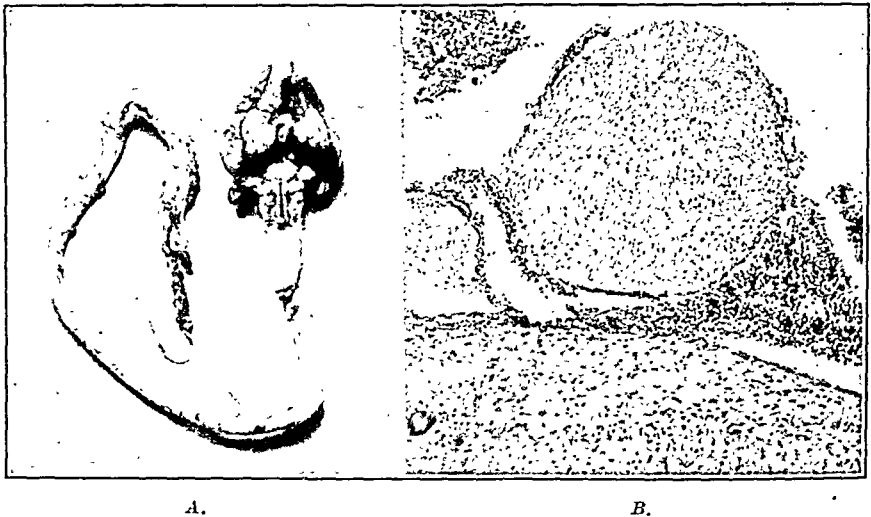
Fig. 4.—A, brain and spinal cord from rabbit injected with the cultures from the teeth of a patient suffering from an acute facial paralysis. Note the hemorrhage and infection at the base of the brain and in the caudal end of the cord. B, photomicrograph of section from base of brain shown in A. Note the exudate around the cranial nerve.

sermann test was negative. Radiographs of the teeth showed that the upper left cuspid (Fig. 5-B), and lower left second bicuspid were pulpless with poor canal filling and some bone absorption at the root tip. The lower left lateral incisor was a peg tooth, the root was poorly filled and there was a large alveolar abscess. The three pulpless teeth were extracted. The brain agar cultures showed no growth from the lower left lateral incisor, and only a few colonies from the lower left second bicuspid. The culture of the upper left cuspid showed an infinity of colonies of a nonhemolytic streptococcus. (Fig. 5-A-C.)

Animal Inoculations.—March 1, 1922, two rabbits were injected with the broth culture from the upper left cuspid. One developed numerous patches of choroidoretinitis and died three weeks later. The autopsy findings were ascites and very large white kidneys. The other developed exudate in the anterior chamber and corneal opacities of both eyes twenty-four hours after inoculation. The following day the eyeballs were extremely red (Fig. 6-A). The right iris was discolored all the way around and the left partly around with milkish grey exudate (Fig. 6-B). There were greyish deposits on the cornea. The animal was killed. The autopsy was negative except for the eye findings. The streptococcus

was recovered by smear and culture from both eyes. March 5, two more rabbits were inoculated with the streptococcus recovered from the left eye of the preceding rabbit. One developed circumcorneal infection and a choroidoretinitis and died thirteen days later. Autopsy showed only kidney abscesses. The other developed a very marked injection of both eyes and died within twenty-four hours. A short chain streptococcus was recovered from the eye. Two rabbits injected with this culture died in a few hours without showing any localized lesions. Two rabbits injected with the culture from the right eye of one of the second set of rabbits, developed patches of choroidoretinitis and were killed six weeks later. One showed no lesions, the other only a purulent arthritis.

Three weeks after the original apical cultures had been made, organisms were removed from the agar tube (Fig. 5-A) with a sterile pipette, grown in broth for twenty-four hours, and injected into three rabbits. One developed a cloudy vitreous and died four days after

Fig. 5.—A, original culture tube from tooth shown in B. C, photomicrograph of streptococcus culture tube at left.

inoculation. Autopsy revealed an arthritis. The second animal showed a pericorneal injection three days after inoculation. The iritis gradually cleared. The vitreous of the right eye became increasingly hazy. The red reflex was lost entirely and there was little pupillary light reflex (Figs. 6-C and 7-B). The animal died eight days after inoculation. Necropsy was negative except for the findings. The patient's vision at the present time is 15/100.

Acute Pyelonephritis

CASE 5.—*History.*—W. W., a medical student, age thirty-four, stated that two weeks previously he had suffered from frequent burning urination, hematuria, chills, and fever as high as 102 degrees. The symptoms improved at first under medication but the chills and fever recurred. He had had a similar attack nine years previously. There had been no other illness. The tonsils were cleanly removed. Physical examination was negative. The urine showed gross blood, many pus cells and a short chain streptococcus which was

recovered by culture. One bicuspid tooth (Fig. 8-*A*) held a large inlay under which the pulp had died. There was no area of rarefication at the tip. The tooth was extracted. From the tip a pure culture of a green producing streptococcus (str. fecalis) was obtained.

Animal Inoculations.—Two rabbits were injected with the culture of the streptococcus. One showed at autopsy multiple abscesses in the pyramids and a purulent arthritis (Fig. 8-*B*). The other showed an acute hemorrhagic nephritis and a few endocardial vegetations. The patient's urine returned to normal and there have been no further symptoms.

Duodenal Ulcer

CASE 6.—*History.*—H. A. A., a business man, age forty-four, had several gastric hemorrhages in December, 1922. For six months previous to this he had had indigestion,

A.   B.   C.   D.
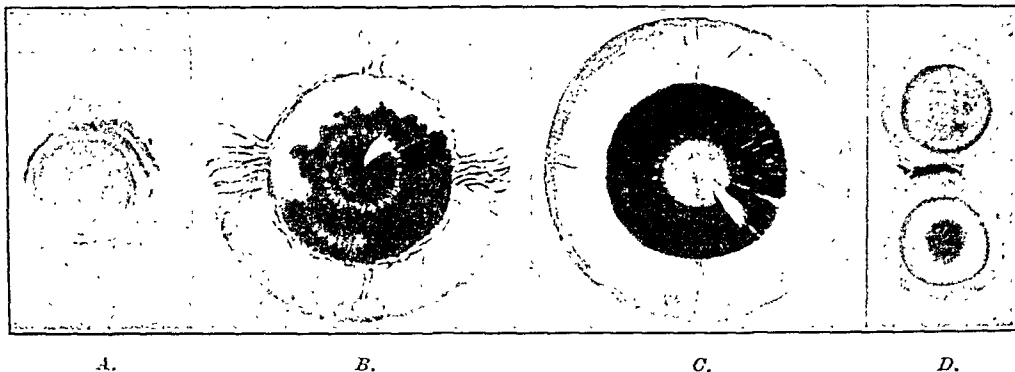
Fig. 6.—*A*, photograph of eye of rabbit twenty-four hours after injection of streptococcus shown in Fig. 5-*C*. *B*, drawing of eye of rabbit injected with same culture. *C*, eye of rabbit several days after injection with attenuated culture from same patient. Note the loss of light reflex due to involvement of vitreous humor. *D*, eyes of rabbit injected with culture from dental foci of same patient one year after the first cultures were made. The eye below is normal, the one above has the anterior chamber filled with purulent exudate.

A.   B.
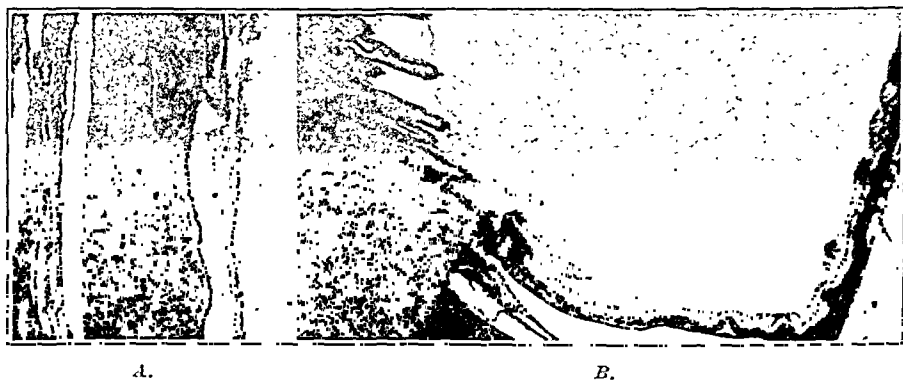
Fig. 7.—*A*, photomicrograph of eye shown in Fig. 6-*B*. Note the cellular infiltration of choroid. *B*, photomicrograph through vitreous humor of eye shown in Fig. 6-*C*. Note the cellular infiltration in vitreous humor.

consisting principally of a feeling of fullness after eating. A diagnosis of duodenal ulcer was made. Several teeth were extracted at this time. There were no further hemorrhages and the symptoms were largely relieved although the patient states that he still has indigestion at times for which he takes soda.

Radiographs in June, 1924, showed one tooth of questionable vitality and one pulpless tooth with little radiographic evidence of infection. At the site of extraction of the upper left bicuspid and first molar some filling material was remaining, and the surrounding bone showed evidence of infection (Fig. 9-*C*). The two teeth were extracted and the infected

bone curetted. Cultures in deep tubes of glucose brain broth agar showed a short chain streptococcus in all.

Animal Inoculations.—Two rabbits were inoculated with the mixed broth cultures. One rabbit was dead the following morning and showed many hemorrhages in the duodenum. The second rabbit was killed. This one showed also many hemorrhages in the first third of the duodenum without lesions elsewhere (Fig. 9-*A*). In order to determine whether the area of infected bone might play a part in the causation of the ulcer one rabbit was injected with 5 c.c. of the broth culture from this area only. At autopsy twenty-four hours later the duodenum showed massive hemorrhages (Fig. 9-*B*). There were no other lesions.

Multiple Onychia

CASE 7.—*History.*—M. K., a housewife, age fifty-one, was first seen in relation to her present infection on January 10, 1922. She gave no history of the acute infectious diseases of childhood. Until three years before she had led an exceedingly active life on a farm. She had had palpitation of the heart and soreness in the chest for many years. At inter-

Fig. 8.—*A*, tooth of patient suffering from an acute pyelonephritis. The pulp had died under the inlay. There was no radiographic evidence of infection. *B*, kidney of rabbit injected with the streptococcus recovered from the tooth shown in *A*. Note the abscesses in the medulla. *C*, photomicrograph through medulla showing an abscess.

vals during the last twenty-three years she had had painful swellings of the larger joints. She had had nycturia two or three times without pain on voiding.

Twelve years before, she began to have trouble with her finger nails. This consisted of swelling, redness and tenderness around the nail roots. At times pus could be expressed. The nails of all the fingers were successively involved, as well as the nails of the great toe on the left foot. The infection usually ended with exfoliation of the nail. There was no history of injury or of the use of irritating substances. The trouble continued until five years before when the patient was referred for dental treatment. Four teeth were found to have periapical abscesses and were extracted. One tooth although pulpless, was negative in the radiograph and was filled and allowed to remain. Following the dental treatment, all the active nail lesions cleared up, as did the arthritis.

On January 10, 1922, the patient returned because she had begun to have pain and redness around one finger nail. She was also having palpitation of the heart. She now had no joint symptoms.

On examination the middle finger of the left hand showed marked swelling and redness around the nail root. The nail was tender on pressure. No pus could be expressed. There was no glandular enlargement. The right tonsil was red and showed a few plugged crypts. The heart action was rapid; the sounds were distinct with a tendency to gallop rhythm; the blood pressure was; systolic, 110; diastolic, 65.

A blood count showed 5,592,000 red cells, 4,950 white corpuscles, and 90 per cent, hemoglobin (normal standard). A differential count of 500 cells showed; polymorphonuclear neutrophiles, 58 per cent; eosinophiles, 1 per cent; basophiles, 0; large mononuclears, 7 per cent; and lymphocytes, 34 per cent. The Wassermann test was negative.

A radiograph of the teeth showed areas of rarefaction around the roots of the lower right molar. All other teeth were vital. There was no pyorrhea.

The lower right second molar was extracted. The nail infection quickly subsided. A urine examination six weeks later showed no albumin or pus cells. The palpitation of the heart disappeared. The patient was seen several months later. There had been no return of the nail infection.

Animal Inoculations.—A culture on blood agar of the roots of the extracted tooth showed a profuse growth of Streptococcus nonhemolyticus 1 (Holman). On January 13,
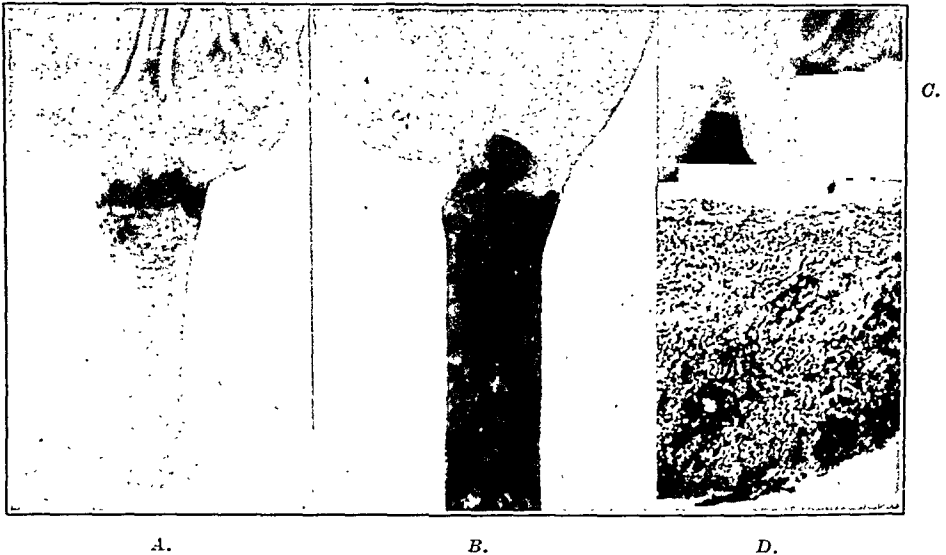
Fig. 9.—*A*, lesions in duodenal bulb produced by the intravenous inoculation of bacteria recovered from areas of dental infection of Patient 6, who was suffering from a duodenal ulcer. *B*, duodenum of rabbit following the intravenous injection of streptococcus recovered from the infected bone (*C*) from same patient. *C*, infected bone at site of extraction of teeth two years previously. Note the small pieces of filling material remaining. *D*, photomicrograph through lesion in duodenum.

one rabbit was injected intravenously with a broth culture of the organism. Four days later a second injection was given. Eight days after the first injection the animal was killed. The postmortem examination showed hemorrhages in the lumbar muscles, a few small abscesses in the cortex of the kidneys and purulent fluid in the shoulder joints. The organism was recovered in pure culture from the joint fluid. Around the nail root of the second toe of the left fore foot there was marked swelling and injection (Fig. 10-*A*). There was hemorrhage around the nail roots of the other toes of the same foot. The other toes were normal. A drawing of the section of the toe which revealed grossly the most marked change shown in Fig. 10-*B*. The nail was removed before the section was cut. The area of polymorphonuclear leucocyte infiltration is evident (Fig. 10-*C*).

The organism recovered from the joint of the first rabbit was injected into two others. These showed muscle hemorrhage and a purulent arthritis and abscesses of the kidney but
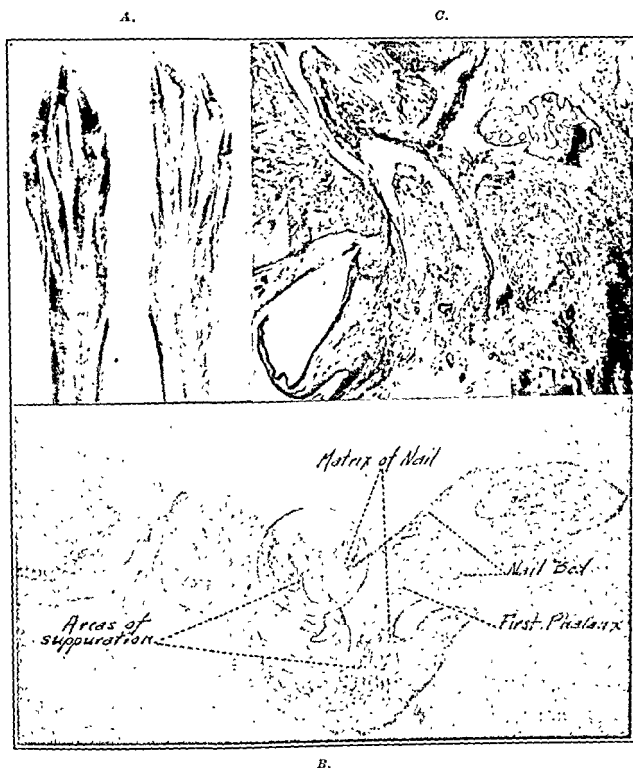
Fig. 10.—A, toe of rabbit following the injection of streptococcus from patient who was suffering from multiple onychia. B, drawing of section of toe after the nail was removed. C, photomicrograph of area at base of nail. Note the areas of polymorphonuclear infiltration.

no involvement of the nails. The organism was carried through two more sets of rabbits with similar results.

SUMMARY AND CONCLUSIONS

I should like to summarize the points which I have attempted to emphasize in this paper as follows:

A very high percentage of teeth, which are negative in the radiograph, harbor infection. The radiograph should never be depended on to eliminate a tooth as a possible focus of systemic disease.

A fairly large percentage of teeth which were positive in the radiograph did not harbor any infection or sufficient infection to be a factor in systemic disease at the time the culture was taken. In such cases, the in-

fection has probably run its course and become bacteria free, just as happens in infections elsewhere in the body.

The periapical tissues of a certain percentage of pulpless teeth, either positive or negative in the radiograph, are sterile when cultured in glucose brain broth. This does not prove that such are really sterile, since some other method of culture might reveal organisms. The findings suggest, however, that a pulpless tooth is not necessarily infected.

The bacteria concerned in chronic foci are quite pathogenic, as judged by their ability to produce lesions in animals on intravenous injection.

In selected cases, one can prove an unmistakable tendency of bacteria from chronic foci to localize in certain parts of the body. These cases afford valuable experimental proof of a causal relation of a chronic focus to systemic disease.

REFERENCES

[1]Haden, R. L.: Arch. Int. Med., 1923, xxxii, 828.
[2]Hartzell, T. B., and Henrici, A. T.: Jour. Nat. Dent. Assn., 1916, iii, 334.
[3]Haden, R. L.: Arch. Int. Med., 1925, xxxv, 457.
[4]Haden, R. L.: Am. Jour. Med. Sc., 1925, clxix, 407.
[5]Haden, R. L., and Jordan, W. H.: Arch. Dermat. and Syph., 1923, ix, 31.
[6]Price, W. A.: Dental Infections, Cleveland, 1923, Penton Publishing Co.

THE PARATHYROID GLANDS AND THEIR IODINE CONTENT*

BY A. M. HJORT, M.D., O.M. GRUHZIT, M.D., AND A. G. FLIEGER, B.S.,
DETROIT, MICHIGAN

PERIODICALLY during the past twenty-five years, articles have appeared in the literature relative to the presence of iodine and its significance in the parathyroid glands. The bulk of the evidence at hand is clean cut, and yet confusion exists, due in part, at least, to an incomplete knowledge of the comparative anatomy of these glands in different species, and to a failure in discriminating between the internal and external glands. To further complicate matters, there appears under parathyroid preparations in *New and Nonofficial Remedies*, a description of the desiccated gland, which includes the iodine content. It is true that these bodies may contain a trace of iodine, but the fact as thus conveyed naturally may lead those unacquainted with the collection of material for the manufacture of parathyroid preparations to conclude that it is contaminated with thyroid tissue. Under the above-mentioned description it is definitely stated that the product represents the external bovine glands. In this species the external glands are distinct, and separated by a considerable margin from the thyroid glands. Contamination by the thyroid in this preparation is, therefore, improbable. At this time it seems pertinent to call to mind the facts revealed by past publications and the observations of the present writers.

IODINE CONTENT OF THE THYROIDS AND PARATHYROIDS

Baumann,[1] in 1896, by adapting a colorimetric method of analysis originally devised by Rabourdin,[2] discovered that the thyroid glands contained a considerable quantity of iodine. This method consisted essentially in the ashing of desiccated gland substance in an alkaline potassium nitrate medium, with subsequent acidification and extraction with chloroform, which yields a violet color, and comparing the color with that developed by a known amount of iodine in a similar mixture.

Gley[3] (1897), by means of this method, reported that the parathyroid glands of rabbits contained more iodine than the thyroids. He used this information in evolving the theory that the parathyroids were an embryonic form of thyroid tissue. Gley's findings were not corroborated by other observers for Chenu and Morel[4] (1904) found but very slight traces of iodine in the external parathyroids of dogs, rabbits, and chicks; and Estes and Cecil[5] (1907) could recover no iodine from the parathyroids of the cow, sheep, and man, and from none to infinitesimal amounts in the dog, and none to 0.06 mg. in three grams of desiccated glands of the horse. As the same method of analysis was used in all of these reports, it is possible that some of

The parathyroid material examined consisted of 43 external bovine glands collected from freshly slaughtered steers, and 109 such glands as collected commercially and frozen for preservation. No difficulty was encountered in collecting the glands, nor in distinguishing them from the neighboring lymph glands in this species. Macroscopically, the glands varied in color from a light tan to dark brown, but in all a yellowish tinge prevailed; they were firmer in consistency and more opaque than the lymph glands; they were
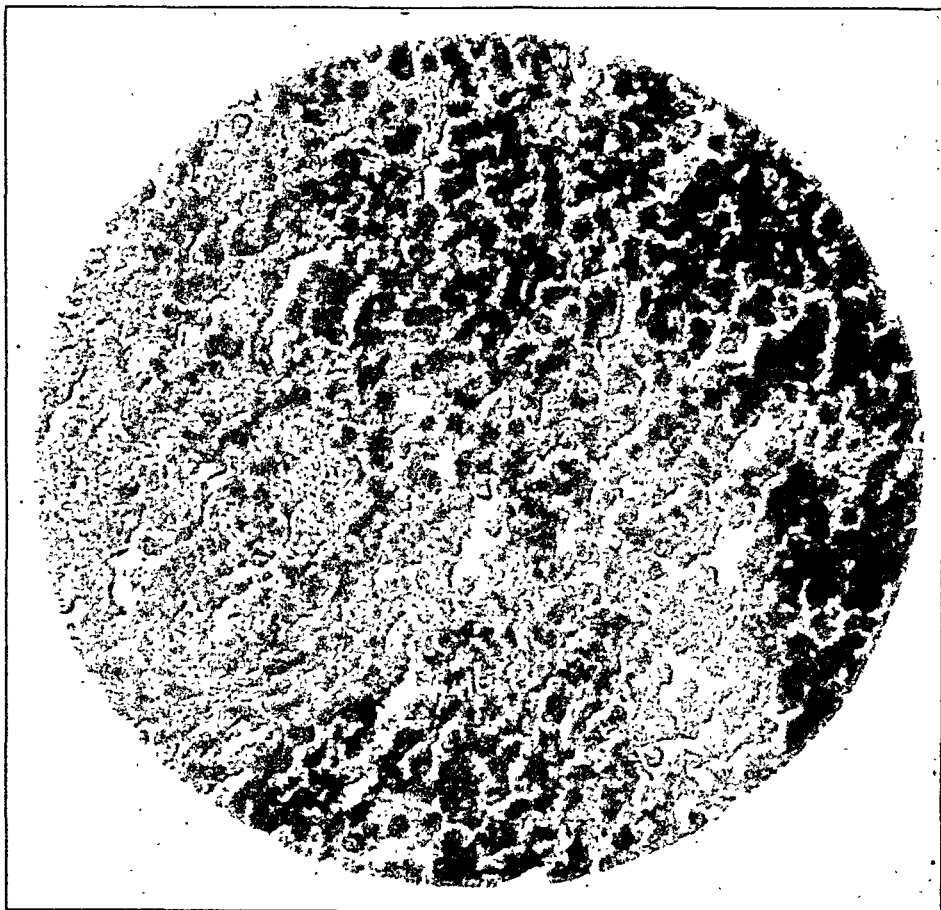
Fig. 3.—Lymph gland. High power. 4 mm. objective, 1X ocular, 24" bellows length.

easily identified by examination of a cut surface, if some doubt was entertained by external appearances. Microscopically, the parathyroid glands showed a distinct structure very different from that possessed by the lymph glands, as shown in Figs. 1, 2 and 3.

From four to eight sections were made at several levels of each gland examined. In none was found thyroid tissue, either intraglandularly or adherent to the capsule. The glandular structure varied somewhat in the different specimens, but only in the relative amount of glandular, as compared

with connective tissue. In none of the glands examined was there found the colloid substance described by Schaper.[17] The glands we collected contained more lymphoid tissue adherent to the capsule than did the commercial product. These observations serve to show that there is no danger of contaminating parathyroid preparations with thyroid tissue at the time of collection from the animal.

THE IODINE CONTENT OF VARIOUS GLANDS

Specimens of desiccated parathyroid, pituitary, thymus, and ovary were chosen for analysis. The first two were obtained from the ox and were desiccated with acetone, the last two were obtained from the calf and hog respectively and desiccated without the assistance of acetone.

The Hunter[16] method of iodine determination has been used by one of us for some years in estimating the iodine content of desiccated thyroid preparations and found to be very reliable and accurate. This method consists essentially of ashing in an alkaline fusion mixture (sodium carbonate, potassium carbonate, and potassium nitrate), with subsequent oxidation of the resulting iodides into iodates, and estimation of the iodine by iodimetry. Since, in our work the amount of iodine was expected to be small, greater care in the selection of reagents, and in the titration was necessary. All reagents were of the highest purity obtainable and were tested for their iodine content before using. Iodine was absent in all, as determined by the silver precipitation test with subsequent solution of the silver chloride with ammonia. A special one cubic centimeter burette graduated into hundredths of a cubic centimeter was used for the titrations. Blank determinations of the reagents used were made after repeating all performances necessary in the preparation of the gland substances for the iodimetric analyses. The blank titrated constantly at 0.1 c.c. of N/200 sodium thiosulphate.

The results of analysis of the various glands above mentioned are included in Table I.

TABLE I

GLAND	WEIGHT DESIC-CATED GLAND IN GM.	$Na_2S_2O_3$* c.c. N/200	IODINE MG.	IODINE %
Parathyroid	2	0.15	0.09511	0.00476
Parathyroid	2	0.10	0.06341	0.00317
Parathyroid	2	0.20	0.12682	0.00634
Ovary	2	0.10	0.06341	0.00317
Pituitary	2	0.15	0.09511	0.00476
Thymus	2	0.10	0.06341	0.00317

* c.c. N/200 $Na_2S_2O_3$ represents the amount after deduction of 0.1 c.c. for the blank.

The accuracy of this method when used in the estimation of as minute amounts of iodine as is contained in the body tissues, other than the thyroid, is limited. The end point in the titration cannot be determined within 0.05 c.c. However, all of the analyses were in such close proximity to each other as to warrant the conclusion that but a very slight trace of iodine is contained in the glands examined and that the iodine is quite uniformly dis-

tributed throughout the animal glandular system, the thyroid excepted. These findings are essentially a corroboration of the majority of past observations.

CONCLUSIONS

1. Parathyroid preparations made from the external bovine glands were not contaminated with thyroid tissue.

2. The iodine content of the parathyroids is of no greater magnitude than that of the ovaries, pituitary and thymus.

3. The iodine found in these glands probably represents the normal distribution of that substance throughout the body tissues.

REFERENCES

[1]Baumann, E.: Ztschr. f. physiol. Chem., 1896, xxi, 319.
   Baumann, E., and Ross, E.: Ibid., 1896, xxi, 481.
   Baumann, E.: Munchen. med. Wchnschr., 1896, xliii, 309.
[2]Rabourdin: Ann. D. Chem. u. Pharm., 1850, lxxvi, 375.
[3]Gley, E.: Compt. rend. Acad. d. sc., 1897, cxxv, 312.
[4]Chenu, J., and Morel, A.: Compt. rend. Soc. de biol., 1904, lvi, 680.
[5]Estes, W. L., and Cecil, A. B.: Bull. Johns Hopkins Hospital, 1907, xviii, 331.
[6]Baumann, E.: Ztschr. f. physiol. Chem., 1896, xxii, 11.
[7]Miwa, and Stöltzner: Jahrb. f. Kinderh., 1897, xlv, 87.
[8]Mendel, L. B.: Am. Jour. Physiol., 1900, iii, 285.
[9]Oswald, Ad.: Ztschr. f. physiol. Chem., 1897, xxiii, 265.
[10]Schnitzler, J.: Wien. klin. Wchnschr., 1896, ix, 657.
[11]Cunningham, R. H.: Jour. Exper. Med., 1898, iii, 148.
[12]Wells, H. G.: Jour. Biol. Chem., 1909, vii, 259.
[13]Denis, W.: Ibid., 1911, ix, 363.
[14]Riggs, L. W.: Jour. Am. Chem. Soc., 1909, xxxi, 710.
[15]Seaman, E. C.: Jour. Biol. Chem., 1920, xliii, 1.
[16]Hunter, A.: Ibid., 1909, vii, 321.
[17]Schaper: Arch. f. mikr. Anat. u. Entwkl., 1895, xlvi, 95.

NARCOTIC AND TOXIC POTENCY OF ALIPHATIC ALCOHOLS UPON RABBITS*

By J. C. Munch, Ph.D., and E. W. Schwartze, A.B., M.D., Washington, D. C.

INTRODUCTION

THE purpose of the investigation here reported was to determine the toxicity of the lower aliphatic monatomic alcohols when administered by mouth. Such data were not available for some of the alcohols studied. Moreover, the propriety of comparing the results of different investigators studying different alcohols by different methods is at times questionable. Some of the other criteria for comparing and studying the alcohols pharmacologically are purely arbitrary and are, therefore, not directly indicative of oral toxicity ratios. Intravenous administration may cause emboli. Neither the adequacy nor the necessity of hirudin in such injections has been shown. In subcutaneous administration, absorption is usually poor or accompanied by necrosis of a great deal of tissue. Intraperitoneal administration† is impracticable because solutions for injection containing comparable doses of the different alcohols either cannot be prepared on account of their insolubility in suitable media or, if prepared, are too bulky.

REVIEW OF LITERATURE

The earliest investigation of the narcotic and toxic effects of a series of homologous alcohols reported, is that by B. W. Richardson.[46] In studying the relation between molecular weight and physiologic action he deduced the so-called "Richardson Law," "Weight, caeteris paribus, intensifies action and makes it more prolonged." The following year, Rabuteau[44] concluded from his experiments that "the alcohols of the series $C_nH_{2n} + {}_2O$ are the more active as the group CH_2 enters a larger number of times into their structure." Since the time of these two investigators, numerous experiments in which various forms of intact plant[21, 38, 50, 51] and animal[3, 4, 9, 18, 25, 42, 57] life, surviving organs,[22, 30, 59] nerves,[8, 17, 24, 45, 55] muscles,[5] enzymes,[36, 56] and even physicochemical experiments[28, 49] were employed, have revealed no more important or acceptable quantitative pharmacologic generalization. The mechanism of action, both on the unicellular and multicellular organisms, however, is better understood qualitatively. Mathematical discussions of the toxicity of alcohols are still a matter of dispute and the mechanism of pharmacologic action is still a fruitful field for investigation.††

TABLE II—CONT'D

MOLAR TOXICITY OF ALIPHATIC ALCOHOLS

INVESTIGATOR	METHOD OF STUDY	TEST ANIMAL OR SUBSTANCE	MEOH	ETOH	PROH	ISO-PROH	N-BUOH	ISO-BUOH	SEC-BUOH	TERT-BUOH	N-AMOH	ISO-AMOH*	SEC-AMOH	TERT-AMOH	REMARKS
(7)	"	Fish	0.69 / 0.5000	1.00 / 0.3430	1.3 / 0.2660	1.3 / 0.2660	31.80 / 0.0108			31.8 / 0.0108		70.2 / 0.0045			"Strength" considered volume concentration
(10)	"	Av. on 6 types fish	0.77 / 0.2830	1.00 / 0.2170	4.6 / 0.0472			13.2 / 0.0164				35.0 / 0.0062			Death within 24 hr.
(3)	"	Trout		1.00 / 0.7720	3.2 / 0.2430			7.8 / 0.0984							
(34)	Immersion	Gudgeon	0.55 / 1.25	1.00 / 0.6870	2.0 / 0.3370			3.1 / 0.2190				5.0 / 0.1380			Kill at once (?)
(9)	"	Fundulus heroclitus	0.83 / 1.50	1.00 / 1.25				20.8 / 0.06				50.0 / 0.025			Concentration to stop gill arches in approx. 15 minutes.
(9)	"	Starfish eggs	0.53 / 3.75	1.00 / 2.0				8.0 / 0.25				25.0 / 0.08			Concentration to cause liquefaction.
(18)	"	Sea urchin eggs (Psammechinus miliaris)	0.33 / 0.94	1.00 / 0.31	4.0 / 0.078										Checks multiplication & division of eggs of Psammechinus miliaris.
(19)	"	Stronglyocentrotus lividus	0.57 / 0.719	1.00 / 0.408	3.0 / 0.136		9.0 / 0.0454					20.0 / 0.0204			
(21)	"	Convoluta	0.32 / 0.938	1.00 / 0.304	3.4 / 0.090		11.2 / 0.027								
(4)	"	Paramecia	0.46 / 1.225	1.00 / 0.567	2.8 / 0.204	2.0 / 0.280	7.4 / 0.0765	5.8 / 0.0984							
(13)	Perfusion	Frog heart	0.75 / 1.0	1.00 / 1.33	1.5 / 2.0		4.5 / 6.0					26.3 / 35.			To stop heart. Ratios: MeOH = 1.0.
(31)	Perfusion	Rabbit heart	1.00 / 1.0	1.00 / 1.0	5.0 / 5.0		7.5 / 7.5					75.0 / 75.0			To stop heart—Ratios MeOH = 1.0.
(32)	"	Rabbit intestine	0.80 / 1.0	1.00 / 1.25	9.8 / 12.25		36.0 / 45.0					108.0 / 135.0			To inhibit contraction.
(47)	"	Frog heart	0.56 / 0.56	1.00 / 1.00	1.9 / 1.92	2.6 / 2.56		6.7 / 6.68		2.8 / 2.82		17.1 / 17.1			Ratio:—EtOH = 1.

Table II—Cont'd

INTESTIGATOR	METHOD OF STUDY	TEST ANIMAL OR SUBSTANCE	MEOH	ETOH	PROH	ISO-PROH	N-BUOH	ISO-BUOH	SEC-BUOH	TERT-BUOH	N-AMOH	ISO-AMOH[*]	SEC-AMOH	TERT-AMOH	REMARKS
(54)	Immersion	Tortoise heart	0.50 / 0.106	1.00 / 0.0530	2.8 / 0.0188	2.2 / 0.0235	13.1 / 0.0040	11.2 / 0.0047	8.5 / 0.0062	3.7 / 0.0144		31.6 / 0.0017		7.41 / 0.0071	Ratio: MeOH = 1.0.
(55)	Immersion	Various muscles	0.50	1.00											
(8)		Motor nerves	1.0	2.0											
(23)	Hemolysis	Ox R B C	1.0 / 0.44	1.0 / 1.00	10.0 / 3.0		10.2 / 0.318					20.0 / 35.6			Mol. per liter to hemolyze in 5 min.
(49)	""	""	7.31 / 0.48 / 8.6	3.24 / 1.00 / 4.10 / 1.00	1.08 / 2.9 / 1.40	1.7 / 2.80 / 2.0 / 2.13						0.091 / 23.4 / 0.175	6.1 / 0.67		"
(51)	Plasmolysis	Red onion cell (April)	0.48 / 10.0	4.84 / 1.00	2.8	1.7 / 2.80									Mol. per liter which just permit plasmolysis.
(51)		(August)	0.47 / 9.12	4.27 / 1.00	1.50	2.0 / 2.13 / 2.8 / 35.67									
(52)	Hemolysis	R B C ox, dog, man, rabbit	1.00+ / 100.+	100.				5.6 / 17.89				15.3 / 6.54			No hemolysis in 3 hrs. (as ratios).
(53)	""	R B C ox	1.00 / 100.	1.00 / 100.	2.1 / 47.00		6.9 / 36.0	3.4 / 29.0	8.5	2.8 / 31.8	35.6 / 62.2	8.0 / 12.5		6.1 / 7.4	"
Range of molar values:			0.33–1.00	1.30–9.8	1.30–2.8	1.3–2.8	6.9–36.0	3.1–20.8	8.5	2.8–31.8	35.6–62.2	5.0–108.0		6.1–7.4	

The lower figure of each set is the absolute molar value of the alcohol in question, required to produce the designated result; the upper figure indicates the relative molar potency when the absolute value for ethyl alcohol is taken *as a unit*.

[*] Many investigators report results on amyl alcohol; unless further identified by chemical constants, the assumption has been made that they were really using isoamyl alcohol.

A résumé of the literature on the comparative narcotic and toxic action of various alcohols found in the course of this investigation is given in Tables I and II. The data are presented on the molar basis (gram-molecules per liter of solution), the customary method of chemical comparison. They may be readily converted into grams or cubic centimeters by reference to physical constants in Table III. The outstanding feature is the increase in toxicity with increase in molecular weight.

Several investigators have reported that methyl alcohol is more[5, 16] or is less[8, 13, 17, 19, 33, 45, 55, 57] toxic than ethyl alcohol using acute criteria. Besides the above-mentioned investigations, textbooks and some of the literature leave the impression that methyl alcohol is more toxic than ethyl alcohol. Examination shows that this deduction is based upon the size of doses producing any untoward effect, regardless of the fact that the criteria may be dissimilar. The merits of each point of view are discussed later in this article. Studies have also been made in an effort to learn the reason why methyl alcohol, which is less toxic than ethyl alcohol when the production of death within the period of forty-eight hours is used as a criterion, is more toxic than ethyl alcohol when given in small doses over a period of time. The method of excretion, a subject still under investigation, seems to be responsible for this difference. Some investigators have concluded that any member of a homologous series is three times as toxic as its predecessor,[28, 49] but this exactness has been questioned in later work.[21, 53]

Iso-alcohols are usually considered to be less toxic than the normal straight-chain alcohols containing the same number of carbon atoms.[26, 29, 30, 49, 50, 51, 54] Tertiary alcohols are more completely narcotic than secondary alcohols and both are more so than primary alcohols.[26, 28, 29, 48] Temperature increases the toxicity and the narcotic action upon fish.[37] Intramuscular injections of methyl and ethyl alcohol into dogs and rabbits were reported to be a little more toxic than intravenous injections.[27] This conclusion, however, is based on relatively late deaths, not on experiments in which death followed promptly from the intravenous injection of relatively large doses. Other investigators[11, 15] have found subcutaneous injections to be less toxic than intravenous, intraperitoneal, or oral administration.

EXPERIMENTAL PROCEDURE

Alcohols.—The alcohols used in this investigation were procured from various commercial firms* as special C. P. alcohols. The boiling point and density of each was determined in the Analytical Reagent Investigations Laboratory† of this Bureau as soon as the products were received. The refractive indices at 25° C. were determined by one of us for another research (Table III). In most cases these constants agreed closely with data given in the literature and indicate that most of the alcohols were of a high degree of purity.

Rabbits.—The rabbits used in this investigation were bought on the open

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TABLE III

Physical Constants of Alcohols Used

ALCOHOL	BOILING POINT AT 760 MM	DENSITY	INDEX OF REFRACTION AT 25° C. ND	FORMULA	MOLECULAR WEIGHT	RATIO OF MOLECULAR WEIGHT ETOH—1.0
Methyl	66.4*	0.7976 at 15	1.3275	$H—CH_2OH$	32.037	0.69
Ethyl	77.9*	0.7948 at 15	1.3595	$CH_3—CH_2OH$	46.058	1.00
n-Propyl	97-98	0.8126 at 15	1.3820	$CH_3CH_2—CH_2OH$	60.079	1.30
Isopropyl	80.9	0.7976 at 25	1.3768	$\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}{>}CHOH$	''	1.30
n-Butyl	116.6	0.8089 at 25	1.3970	$CH_3CH_2CH_2—CH_2OH$	74.100	1.61
Isobutyl	106.0	0.8068 at 15	1.3936	$\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}{>}CH—CH_2OH$	''	''
sec-Butyl**	98.0	0.8108 at 16	1.3942	$\begin{smallmatrix}CH_3CH_2\\CH_3\end{smallmatrix}{>}CHOH$	''	''
tert-Butyl	82.0	0.7859 at 25	1.3840	$\begin{smallmatrix}CH_3\\CH_3—C—OH\\CH_3\end{smallmatrix}$	''	''
Isoamyl	130.1	0.8095 at 25	1.4042	$\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}{>}CHCH_2—CH_2OH$	88.121	1.91
sec-Amyl**	119.0	0.8091 at 25	1.4050	$\begin{smallmatrix}CH_3CH_2\\CH_3\end{smallmatrix}{>}CHOH$	''	''
tert-Amyl	101.5	0.8134 at 15	1.4020	$\begin{smallmatrix}CH_3CH_2\\CH_3—C—OH\\CH_3\end{smallmatrix}$	''	''

* Corrected for barometric pressure.
** Racemic mixtures.

market from dealers in or near the District of Columbia. No attempt was made to segregate the animals according to weight, color, or sex. All the animals used weighed over 1000 grams, most of them weighing between 1500 and 2500 grams. Darenberg[12] found that rabbits weighing more than 2400 grams were often extremely resistant to intravenous injections of alcohol.

Upon receipt in the laboratory all rabbits were weighed, tagged, and placed in hutches. They were fed a mixture of alfalfa hay and oats daily, and carrots several times a week. Usually animals were held for a fore-period of ten days or two weeks before use, in order to make sure that they were in good condition. They were usually weighed three times a week and those in poor condition, as indicated by consistent loss in weight, were not used.*

Method of Administration.—The rabbits were weighed on the morning of an experiment and injections were usually made between 9 and 11 A.M. For the first few experiments about 50 c.c. of diluted aqueous alcohol solution per kilo body weight was injected. Subsequently the desired dose of alcohol was added to a sufficient volume of normal saline solution to make a total of 50 c.c. immediately before injection, whether or not complete solution could be obtained.

A vaselined rubber catheter (size 14 or 15 French) was used as a stomach tube. It was passed into the stomach of the rabbit and the diluted alcoholic solution or mixture was allowed to run into the stomach, either by gravity through a funnel which was immediately washed with a small quantity of

*Animals were used which were in good condition, since Mansfield[40] observed a difference in narcotic dose depending upon whether starved or well nourished animals were used. We have not investigated this phase.

water to wash in all the alcoholic solution or, later, forced directly into the stomach tube from a 50 c.c. record syringe.

Classification of Pharmacologic Effect.—Immediately after injection, the stomach tube was removed and the animal was placed on a large table. The time of injection and the appearance of symptoms grouped in accordance with the following criteria, were carefully recorded.

(1) *Staggering or Excitement.*—This stage was arbitrarily taken as the first stage of effect. The animal usually roamed wildly about (incoordination), and at times would plunge and attempt to jump from the table to the floor. The respiration and pulse were rapid.

(2) *Light Narcosis.*—Light narcosis followed the first stage when a large enough dose had been given. The animal usually sank into a stupor, ceased to move, and rested on its side or chest, making few if any voluntary movements. It would, however, respond to manual compression or stimulation of the thigh (muscles or sciatic nerve) by moving or by rising to its feet and moving away, but it would lie down again.

(3) *Profound Narcosis.*—Profound narcosis usually followed very rapidly after light narcosis if large enough doses were given. In this stage the corneal reflexes disappeared and the animal lay motionless, making no responses to a stimulus such as stimulation of the thigh. Occasionally, however, involuntary motions, such as attempted running or involuntary winking, after the disappearance of corneal reflexes, were noticed. Nystagmus was frequently observed. Respiration was diminished and labored. The rate of the heart beat was also diminished.

(4) *Death.*—Animals which had reached the stage of profound narcosis were inspected frequently to determine the approach of death, and all animals were inspected before the laboratory was closed in the evening. To prevent the possibility of death from exposure to low temperatures while narcotized, the laboratory temperature was maintained at about 65° or 70° in the winter by a constant temperature apparatus.

(5) *Recovery.*—After profound narcosis was produced, efforts were made in all cases to determine the first appearance of voluntary movements. If the animal was only in the light narcosis stage, the first appearance of an ability to assume a standing position was noted. In each case an attempt was made to determine when the animal was practically normal, as judged by its appearance, respiration, color of skin, powers of motion, and readiness to eat carrots, apples, or similar appetizing food.

RELATIVE MOLAR NARCOSIS AND TOXICITY

Basis of Comparison of Action of Alcohols.—Most of the data given in the literature deal with the production of death. In the investigation here reported, the quantities of the alcohols required to produce narcosis and to produce death within a period of approximately twenty-four hours were determined.

The minimum narcotic dose (M N D) was taken to be that dose by which light narcosis was produced in about half of the animals. For instance,

where injections of 5 c.c. of ethyl alcohol per kilo produced no narcosis and injections of 6 c.c. per kilo, the next larger dose, produced narcosis in all test animals fairly rapidly, the M N D was half way between these doses, 5½ c.c. per kilo. The certain lethal dose (C L D) was the dose which would produce death in about twenty-four hours in practically all animals injected. When a given dose produced death in only a portion of the animals within twenty-four hours and the next higher dose injected produced death in all animals, or practically all the animals, in half a day or less, that quantity half way between was arbitrarily selected as the C L D.

EXPERIMENTAL RESULTS AND DISCUSSION

The M N D and C L D are given in Table IV. The average values in cubic centimeters per kilogram are given in the second and third columns; the corresponding molar values are given in the next two columns. To facilitate comparisons of narcotic or toxic doses, the ratios of molar potency have been calculated, taking the absolute molar values of ethyl alcohol (0.0945 mol. per kilogram as the minimum narcotic dose and 0.2150 mol. per kilogram as the certain lethal dose) as units. The last column shows the ratios obtained by dividing the C L D by the M N D, arranged according to the alcohol group.

TABLE IV

NARCOTIC AND TOXIC POTENCIES OF ALIPHATIC ALCOHOLS. ORAL ADMINISTRATION TO RABBITS

ALCOHOL	AVERAGE C.C. PER KG.		MOL. PER KG.		RATIO OF MOLAR POTENCY ETOH —1.00		RATIO $\frac{CLD}{MND}$			
	MND	C					N-PRI-	ISO-PRI-	SECONDARY	TERTIARY
Methyl	7.5									
Ethyl	5.5	1								
n-Propyl	1.75									
Isopropyl	2.85									
n-Butyl	1.05									
Isobutyl	1.75									
sec-Butyl	1.25									
tert-Butyl	1.80									
Isoamyl	0.875									3.3

MND—Minimum Narcotic Dose.
CLD—Certain Lethal Dose.

As the molecular weight increases, the narcotic and toxic properties increase; the narcotic properties for most alcohols increased more rapidly than the toxic properties. As the homologous series of normal, iso-primary, secondary, or tertiary alcohols, is ascended, the lethal dose in any series becomes a greater multiple of the narcotic dose, with the exception of methyl and normal propyl alcohol. Methyl alcohol has always been an exception in making generalizations,[5, 55] both chemical and pharmacologic. Normal propyl alcohol is the only primary alcohol with which we are familiar that is miscible in all proportions with both oil and water.

Experiments with iso-alcohols have been very limited. Because "isopropyl" alcohol has the name "iso," is not a criterion that it should be considered in the series with primary isobutyl and isoamyl alcohols. It is a secondary alcohol and being the first member in such a series probably explains why it exhibits irregularities. Therefore, we have results on only one alcohol (isobutyl) suitable for comparison with those obtained on a normal alcohol of the same carbon content. Alone, the data upon this alcohol are not sufficient to warrant drawing definite conclusions. Our results with this lone alcohol show that it is less narcotic but more toxic than its straight chain isomer. This does not agree with expectations.[26, 29] Further work is necessary.

The secondary alcohols show the greatest ranges between their narcotic and toxic doses. These alcohols are more narcotic than the isomeric isoprimary, but less narcotic than the normal primary alcohols used. They are also less toxic than the normal alcohols used.

The data on the tertiary alcohols are insufficient for a prediction on the behavior of the series. Tertiary butyl is about as toxic and narcotic as the isoprimary butyl compounds, whereas tertiary amyl is a little more toxic and narcotic than its corresponding isomer.

Methyl Alcohol.—Deaths from alcohols other than methyl occurred within twenty-four hours in a great proportion of cases. Methyl alcohol intoxication from a single dose is clearly divisible into two classes, (1) acute poisoning, and (2) delayed poisoning, in which, while the animal is not sober, it is more or less completely exhausted. An animal's degree of narcosis as observed in 1, distinctly lessens before it dies in stage 2. Since in our experiments other alcohols have not shown a delayed poisoning of the same type as methyl alcohol, it is manifestly unfair to make a comparison except on the common basis of acute effect. However, in our experiments, irrespective of class, considering *all* deaths, methyl alcohol appears to be as toxic as ethyl on the cubic centimeter basis; on the molar basis, ethyl alcohol would then be, of course, 33 per cent more toxic than methyl.

From the above consideration, which concerns acute intoxication, we do not wish to leave the impression that the other effects are to be minimized. From a practical standpoint any untoward effects whatsoever, chronic or delayed poisoning, blindness, cystitis, etc., are to be considered. The purpose of the comparison, therefore, underlies the analysis, and necessarily narrows the applications of the conclusion. It is hardly necessary to state that alcohols other than methyl, using different experimental conditions, might give results which we could not compare with methyl on account of the lack of a common basis.

<center>CONCLUSIONS</center>

The general statement regarding the increase in toxicity in homologous series with increase in molecular weight has been verified for oral administration.

This law may be extended to narcotic action following oral administration.

Increase in narcotic activity is more rapid than increase in toxicity in an

homologous series of alcohols, when administered orally, some alcohols having greater narcotic potency than might be expected from their molecular weights.

REFERENCES

[1] Antheaume, Andre: Contribution a l'etude de la toxicité des alcools et de la prophylaxie de l'alcoolisme, Thése, Paris, 1897.

[2] Baer, Georg: Beitrag zur kenntnis der acuten vergiftung mit verschiedenen alkoholen, Dissertation, Berlin, 1898.

[3] Billard, G., and Dieulafe, L.: La toxicité des alcools, fonction de leur tension superficielle, Compt. rend. Soc. de biol., 1904, lvi, 452.

[4] Bills, C. E.: A Pharmacological Comparison of Six Alcohols, singly and in Admixture, on Paramecium, Jour. Pharmacol. and Exper. Therap., 1923, xxii, 49-57.

[5] Blumenthal, Arthur: Über die wirkung verwandter chemische stoffe auf das quergestreifen muskel, Arch. f. d. ges. Physiol., 1895-6, lxii, 513-542.

[6] Boruttau, H.: Die verwendung von isopropylalkohol zur hygienischen und kosmetischen zwecken, Deutsch med. Wchnschr, 1921, xlvii, 747.

[7] Bradford, John Buckley: Some Points Connected with Sleep, Sleeplessness, and Hypnotics, Brit., Med. Jour., July 1, 1899, pp. 4-9.

[8] Breyer, Hans: Über die einwirkung verschiedener einatomiger alkohole auf das flimmerepithel und die motorische nervenfaser, Arch. f. d. ges. Physiol., 1903, xcix, 481-512.

[9] Brown, O. H.: A Pharmacological Study of Anesthetics and Narcotics, Am. Jour. Physiol., 1905-6, xv, 85-97.

[10] Cololian, P.: La toxicité des alcools chez les poissons, Jour. de physiol. et de path. gén., 1901, iii, 535-546.

[11] Conte, G.: Contributo sperimentale allo studio della tossicita degli alcools e di alcune essenze commerciali, Ann. di med. nav., 1912, xviii, 514-540.

[12] Daremberg, G.: La toxicité des boissons alcooliques mesurée a l'aide des injections intraveineuses, chez le lapin, Arch. de méd exper. et d'anat. path., 1895, vii, 719-752.

[13] Dold, Hermann: Über die wirkung des äthylalkohols und verwandter alkohole auf das froschherz, Arch. f. d. ges. Physiol., 1906, cxii, 600-622.

[14] Ducceschi, V.: Le coefficient toxique de quelques boissons alcooliques, Arch. ital. d. biol., 1920, lxx, 112-114.

[15] Dujardin-Beaumetz, M., and Audigé, M.: Sur les propriétes toxiques des alcools par fermentation, Compt. rend., 1875, lxxxi, 192-194.

[16] —: Recherches experimentales sur la puissance toxique des alcools, 1879, Paris.

[17] Efron, J.: Beiträge zur allgemeinen nervenphysiologie, Arch. f. d. ges. Physiol., 1885, xxxvi, 467-488.

[18] Führer, Hermann: Über die einwirkung verschiedener alkohole auf die entwicklung der seeigel, Arch. f. exper. Path. u. Pharmakol., 1903, li, 1-10.

[19] —: Pharmakologische studien am seeigeleirn. Der wirkungsgrad der alkohole, Ibid., 1904, lii, 69-84.

[20] —: Nachweis und bestimmung von giften auf biologischen wege, Berlin, 1911, 52-54.

[21] —: Der wirkungsgrad der einwertigen alkohole, Ztschr. f. Biol., 1911-12, lvii, 465-494.

[22] —: Die wirkungsstärke der narcotica. I. Versuch an isolierten froschherzen, Biochem. Ztschr., 1921, cxx, 143-163.

[23] Führer, Hermann, and Neubauer, Ernst: Hämolyse durch substanzen homologer reihen, Arch. f. exper. Path. u. Pharmakol., 1906-7, lvi, 333-345.

[24] Grützner, P.: Über die chemische reizung sensibler nerven, Arch. f. d. ges. Physiol., 1894, lviii, 93-94.

[25] Irwin, Marian: Sensory Stimulation by Saturated Monohydric Alcohols, Am. Jour. Physiol., 1922, lv, 151-154.

[26] Iwanoff, K. S.: Über die wirkung einiger metallsalse und einatomiger alkohole auf die entwickelung von schimmelpilzen, Centralbl. f. Bakteriol. u. Parasitenk., 1904-5, xiii, 139-144.

[27] Joffroy, A., and Serveaux, R.: Nouveau procede de mensuration de la toxicité des liquides par la methode des injections intraveineuses, Arch. de méd. exper. et d' anat. path., 1895, vii, 569-588.

—: Mensuration de la toxicité experimentelle et de la toxicité vraie de l'alcool methylique, Ibid., 1896, viii, 473-509.

—: Mensuration de la toxicité vraie de l'alcool ethylique, Ibid., 1897, ix, 681-707.

[28] Kamm, Oliver: The Relation Between Structure and Physiological Action of the Alcohols, Jour. Am. Pharm. Assn., 1921, x, 87-89.

[29] Kemp, H. P.: Jour. Physiol., 1908, xxxvii, 49.

[30] Kochmann, M.: Die einwirkung des alcools auf das warmblüterherz, Arch, internat. de pharmacol., 1904, xiii, 329-377.

[31] Kuno, Yas.: Über die wirkung der einwertigen alkohole auf das überlebende saügetierherz, Arch. f. exper. Path. u. Pharmakol., 1913, lxxiv, 399-416.

32—: Über die wirkung der einwertigen alkohole auf den überlebenden kaninchendarm, Arch. f. exper. Path. u. Pharmakol., 1914, lxxvii, 206-217.
33Langaard, Alexander: Die giftigkeit des methyl und aethylalkohols, Ztschr. Exp. Path. und Therap., 1913, xiii, 20-36.
34Lesieur, Ch.: Nouvelles recherches sur la toxicité experimentelle des alcools alimentaires, Jour. de physiol. et de path. gén., 1906, viii, 427-441.
35Lillie, R. S.: Antagonism Between Salts and Anesthetics. III. Further Observations Showing Parallel Decrease in the Stimulating, Permeability-Increasing, and Toxic Actions of Salt Solutions in the Presence of Anesthetics, Am. Jour. Physiol., 1912, xxxi, 255-287.
36Linossier, G.: Influence comparee des principaux alcools de fermentation sur l'action des diastases, Compt. rend. Soc. de biol., 1899, xxi, 887.
37—: Influence de la temperature sur la toxicité de l'alcool, Ibid., 1917, lxxx, 584.
38Loeb, J.: Chemische konstitution und physiologische wirksamkeit von alkoholen und säuren, Biochem. Ztschr., 1909-10, xxiii, 93-96.
39Macht, David I.: A Toxicological Study of some Alcohols with Especial Reference to Isomers, Jour. Pharmacol. and Exper. Therap., 1920, xvi, 1-10.
40Mansfield, G.: Inanition und narkose, Arch. internat. de pharmacol., 1905, xv, 467-86.
41Otto, Gustav: Über die wirkung einwertiger alkohole auf forellen und deren dottersackbrut, Ztschr. f. Biol., 1912-13, lix, 165-170.
42Overton, E.: Studien über die narkose, Jena, 1901.
43Picaud, M.: Sur la toxicité des alcools, Compt. rend, 1897, cxxiv, 829-831.
44Rabuteau: De quelques proprietes nouvelles ou peu connues de l'alcools, L'Union Medicale (3), 1870, x, 165-170.
45Raether, Max: Über die einwirkung verschiedener einwertiger alkohole auf sensible nerven und nervenendingungen, Dissertation, Tübingen, 1906.
46Richardson, B. W.: Lectures on Experimental and Practical Medicine. Physiological Research on Alcohols, Med. Times and Gaz., Dec. 18, 1869, pp. 703-706.
47Ringer, S., and Sainsbury, H.: Observations on the Relative Effects of Certain Members of the Ethylic Alcohol Series on the Ventricle of the Frog's Heart, Practitioner, London, 1883, xxx, 339-350.
48Schneegans, A., and Mering, J. V.: Über die beziehungen zwischen chemiker constitution und hypnotischer wirkung, Therap. Monatsh., July, 1892, pp. 327-332.
49Traube, J.: Über die wirkung lipoidlöslicher stoffe auf rote blutkörperchen, Biochem. Ztschr., 1908, x, 371-379.
50Tsukamoto, M.: Über die giftwirkung verschiedener alkohole. Journal the College of Science, Tokio, 1894; through Maly's Jahresb. ü. d. Fortschr. d. Thierchem., 1894, xxiv, 84.
51Vandevelde, A. J. J.: Determination du pouvoir toxique des alcools monatomiques par la plasmolyse, Arch. internat. de pharmacol., 1900, vii, 123-132. Further comment by Errara, L.: Remarques sur la toxicité moleculaire de quelques alcools, Soc. roy. d. sc. méd. et nat. de Brux.: Bull., 1900, pp. 18-31.
52—: Recherches sur les hemolysines chimiques, Bull. Soc. chim. Belg., 1905, xix, 288-337.
53—: Über die bestimmung der giftigkeit chemischer verbindungen durch die bluthämolyze III, Chem.-Z., 1906, xxx, 296-7.
54Vernon, H. M.: The Action of Homologous Alcohols and Aldehydes on the Tortoise Heart, Jour. Physiol., 1911-12, xliii, 325-342.
55Verzar, Fritz: Über die wirkung von methyl und äthyl-alkohol auf das muskelfaser, Arch. f. d. ges Physiol., 1909, cxxviii, 398-420.
56Warburg, O., and Wiesel, R.: Über die wirkung von substanzen homologer reihen auf lebensvorgänge, Arch. f. d. ges. Physiol., 1912, cxliv, 465-488.
57Whitney, David D.: The Relative Toxicity of Methyl and Ethyl Alcohols as Determined by the Rate of Reproduction in Hydatina Senta, Am. Jour. Physiol., 1912, xxx, 463-465.
58Winterstein, Hans: Die narkose in ihrer bedeutung für die allgemeine physiologie, Berlin, 1919.
59Wolff, P.: Zur herzwirkung der alkohole in beziehung zu ihrer chemischen constitution, Biochem. Ztschr., 1922, cxxxii, 480-4.

A STUDY OF TISSUE AUTOLYSIS IN VIVO*

III. Observations Using the Spleen

Edward C. Mason, M.D., and Edward C. Davidson, M.D.,
Assisted by C. W. Matthew, Ph.C.,
Detroit, Mich.

W E have demonstrated[1] that the autolysis of a small amount of liver tissue free within the abdominal cavity is accompanied by definite and constant changes in the animal's blood chemistry. We have also[2] compared the toxicity of normal liver tissue and autolyzed liver tissue by administering saline extracts of such tissues intravenously.

It has been the object of the present work to ascertain whether or not tissues, other than liver, were capable of liberating toxic material of sufficient potency to kill the animal. For this work the spleen was selected as a tissue of choice since its removal is accompanied by such slight physiologic disturbance and it also has the additional advantages of being readily accessible and easy to remove.

METHOD

All operations were performed in the evening under aseptic conditions, using ether anesthesia with a preliminary injection of morphine sulphate ¼ gr. and atropine sulphate 1/100 gr., the animals having been fed from eight to ten hours prior to the operation. Briefly, the operation consisted of clamping off the spleen through its attachment, sectioning the attachment between the spleen and clamp, ligating the cut surface by use of a figure-of-eight suture, weighing the spleen and returning it to the abdomen. The operation usually required from fifteen to twenty minutes, and the spleen varied in weight from 23 to 77 grams.

None of the animals showed signs of surgical shock following the operation, and the following morning there was but very little evidence of trauma. The animals seemed to withstand the operation even better than those in which a portion of the liver was sectioned. Half of the animals of this series died, and such animals displayed all the characteristics of animals dying from liver autolysis. However, death never occurred so soon following operation and the symptoms were considerably less pronounced.

Postmortem examination revealed essentially the same findings as described in the study of liver autolysis. The abdomen usually contained 100 to 300 c.c. of brown colored fluid, but in no case did the fluid appear to be actual blood. A pseudofibrinous exudate was usually present, being different from a true fibrinous exudate in that it was easily removed, leaving a smooth shiny surface. The intestines were generally hyperemic with marked congestion.

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The omentum was firmly wrapped about the sectioned spleen, the omentum being markedly hemorrhagic and discolored. The autolyzing spleen ranged from dull to a bright green in color. The spleen showed the same autolytic changes which accompany liver autolysis, being less firm than normal spleen as well as gas containing. There was a marked loss in weight of the sectioned spleen, such loss ranging from 13 to 20.5 grams.

We realize that our study of six cases is not extensive enough to warrant any sweeping conclusions. However, unexepected conditions have developed which make it necessary to postpone indefinitely any further work on the subject. We have, therefore, thought it advisable to offer the work in its present state of development.

THE RELATION BETWEEN THE WEIGHT OF THE SECTIONED SPLEEN AND THE SIZE OF THE ANIMAL AND THE PERIOD OF LIFE EXPECTANCY

In our observations on liver autolysis we noted that the weight of the liver sectioned did not give any definite indication as to the period of life expectancy. We then expressed the opinion that the length of life following sectioning of a portion of the liver depended upon two factors: (1) the rate of liberation of the toxic material, and (2) the rate of absorption of the toxic substance. Our work with the spleen has led us to the same conclusion, which is based upon Table I.

TABLE I

WEIGHT OF ANIMAL	WEIGHT OF SPLEEN	PERIOD OF LIFE AFTER OPERATION
16.8 K	68 Grams	− − −
11.0 K	23 grams	45 hours
13.1 K	41 grams	36 hours
15.9 K	77 grams	36 hours 25 minutes
21.2 K	56 grams	− − −
23.1 K	42 grams	− − −

BLOOD CHEMISTRY

The following blood constituents were determined: 1. nonprotein nitrogen, 2. urea, 3. amino acid, 4. uric acid, and 5. sugar. The three animals which lived following the sectioning of the spleen did not show any marked changes in their blood chemistry such as occurred in the animals dying from liver autolysis. This point is illustrated by the blood urea and amino acid fractions.

TABLE II

UREA NITROGEN BEFORE AND AFTER OPERATION

DOG		BEFORE	12 HR.	24 HR.	36 HR.	48 HR.	60 HR.	72 HR.
No.	14	21.	16.3	15.4		14		10.7
No.	22	18.2	10.3	10.7	10.7	12.6		10.7

TABLE III

AMINO ACID NITROGEN BEFORE AND AFTER OPERATION

DOG		BEFORE	12 HR.	24 HR.	36 HR.	48 HR.	60 HR.	72 HR.
NO.	14	8.52	6.82	5.98		5.78		5.71
NO.	23	7.45	6.09	7.09		6 90		
NO.	22	7.00	7.00	7.82	7.00	7.00		7.00

We have made complete chemical studies only on one animal which died following the sectioning of the spleen. The animal showed no marked changes in any of nonnitrogenous blood constituents except the amino acid fraction which is represented by the figures in Table IV.

TABLE IV

AMINO ACID NITROGEN BEFORE AND AFTER OPERATION

DOG		BEFORE	12 HOURS	24 HOURS	36 HOURS
NO.	21	7.27	7.78	8.19	DEAD

This one case is not sufficient to warrant a definite conclusion, but it is suggested that either the other nonprotein nitrogen constituents are not increased or that if an increase occurs it does so in the last twelve hours of life.

Analysis of the abdominal fluid present at death occurring from spleen autolysis gave the figures shown in Table V.

TABLE V

SAMPLE		N.P.N.	UREA	URIC ACID	AMINOACID
DOG	21	58.8 − 60.	33.6 − 34.	2.32 − 2.35	
DOG	21	60.6 − 60.6	35.		12.7 − 12.7

It will be noted that the figures do not vary greatly from those which we have previously found associated with liver autolysis.

CONCLUSIONS

1. Autolysis of the spleen free within the abdominal cavity has been accompanied by death in 50 per cent of the animals studied.

2. Death from spleen autolysis has not followed so soon after operation as death from liver autolysis, the earliest death being thirty-six hours after sectioning the spleen.

3. It is suggested that the spleen either does not undergo as rapid autolysis as the liver tissue or that the toxic material liberated is not so potent.

4. Apparently the blood chemistry is but little altered in the animals which survive sectioning of the spleen, while those dying following the operation show some changes.

5. The analysis of the abdominal fluid present at death due to spleen autolysis shows the nonprotein nitrogen constituents are essentially the same as in those cases of death due to liver autolysis.

REFERENCES

1Mason, and Davidson: A Study of Autolysis in Vivo. I. Blood Changes, Physical and Chemical, JOUR. LAB. AND CLIN. MED., May, 1925, x, 622.
2Mason and Davidson: A Study of Autolysis in Vivo. II. A Pharmacologic Study of the Toxic Material, JOUR. LAB AND CLIN. MED. (In press).

THE EFFECT OF POTASSIUM OXALATE ON BLOOD SUGAR DETERMINATIONS*

By HENRY J. JOHN, M.D., CLEVELAND, OHIO

IN our routine work with diabetic patients, I have noticed an occasional low blood sugar figure which seemed out of proportion with the daily blood sugar estimations obtained from the same patient. In searching for a possible reason for this apparent discrepancy, I noticed that this low figure was obtained in those cases in which only a small quantity of blood—from ¾ to 1 c.c.—was taken, as in children. I, therefore, began to suspect that variations in the amount of the potassium oxalate, used to prevent clotting of the blood, might provide the explanation.

I believe that the usual practice in making blood sugar determinations is to put a pinch of oxalate into the test tube which is to receive the blood, the tube being well shaken as soon as the blood is added to insure thorough mixing, and the blood examined as soon as possible thereafter. Our own practice is to make the blood sugar determination within fifteen minutes.

In order to determine the effect of the oxalate on the blood sugar determination, the following experiment was carried out. Two specimens of blood equal in amount were taken from each of two patients and placed in test tubes. To one specimen from each patient, 15 to 30 mg. of potassium oxalate, the amount used in routine work, was added and to the other specimen from 250 to 350 mg. Blood sugar estimations on these mixtures as obtained by the Myers' modification of the Benedict method, gave the results shown in Table I.

TABLE I

SPECIMENS FROM PATIENT Number	SMALL AMT. OXALATE .Blood sugar mg. per 100 c.c.	LARGE AMT. OXALATE Blood sugar mg. per 100 c.c.	VARIATION
1.	122	82	33%
2.	93	60	35%

These variations led me to make a further investigation of the possible effects of varying amounts of oxalate on blood sugar estimations. The following procedure was used: To each of two sets of eight test tubes containing respectively 15, 30, 45, 60, 75, 100, 150, and 200 mg. of potassium oxalate, 2 c.c. of blood was added. The blood added to the tubes in one set was taken from a diabetic patient and to those in the other set from a nondiabetic patient. Each tube was thoroughly shaken three times at intervals of from one to two minutes, after which whole-blood sugar estimations were made by the Myers-Benedict method with the fairly constant results shown in Tables II and III. The ex-

periment was repeated using the Folin-Wu method of blood sugar determination, with the results shown in Tables IV and V. Table VI shows that the results obtained by the Myers-Benedict and Folin-Wu methods were fairly uniform.

These experiments show that the quantity of potassium oxalate used bears a definite relation to the blood sugar determination. As the amount of oxalate is increased, the blood sugar content determination is decreased in both diabetic and nondiabetic cases.

TABLE II

A. BLOOD SUGAR VALUES IN NONDIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Myers' modification of Benedict method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
	15	30	45	60	75	100	150	200	
Case 1	105	97	94	91			60	60	Blood
2	119	116	116	106	104	102	74	53	sugar
3	108	106	106	102	94	92	74	68	mg./100
4	95	95	94		70		60	30	c.c.
5	107	104	98	98		91	77	50	
6	110		104	97	92	87	81	75	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1	7	10	13			43	43	
2	2	2	11	12	14	38	55	
3	2	2	4	13	15	31	37	
4	0	1		26		37	68	
5	3	8	8		15	28	53	
6		5	12	16	21	26	32	
Average Loss	2.8	4.66	9.6	17	16.37	34	48	Per cent

TABLE III

A. BLOOD SUGAR VALUES IN DIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Myers' modification of Benedict method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
	15	30	45	60	75	100	150	200	
Case 1	258	227	212	207	193	192	187	143	Blood
2	348	344	336	313	309	277	224	191	sugar
3	405		394		333		333	263	mg./100
4	157		145		138		97	76	c.c.
5	150	150	148	143	141	132	129	116	
6	161	161	156	156	156	154	143	134	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1	12	18	20	23	26	28	44	
2	1	3	10	11	20	36	45	
3		3		18		18	35	
4		8		12		38	52	
5	0	1	5	6	12	15	22	
6	0	3	3	3	4	11	17	
Average Loss		3.25	6	9.5	12.5	15.5	24.3	36	Per cent
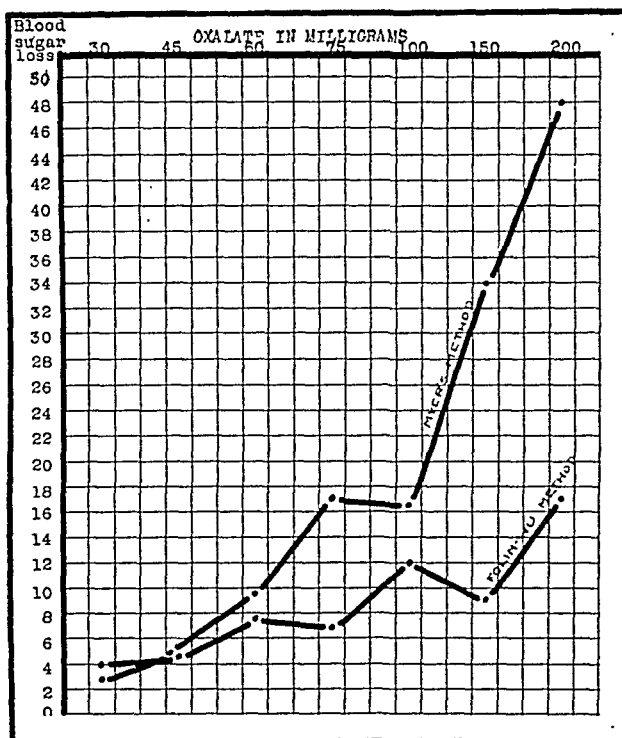
Chart 1.—Percentile decrease in the blood sugar content produced by varying amounts of potassium oxalate. Nondiabetic cases.
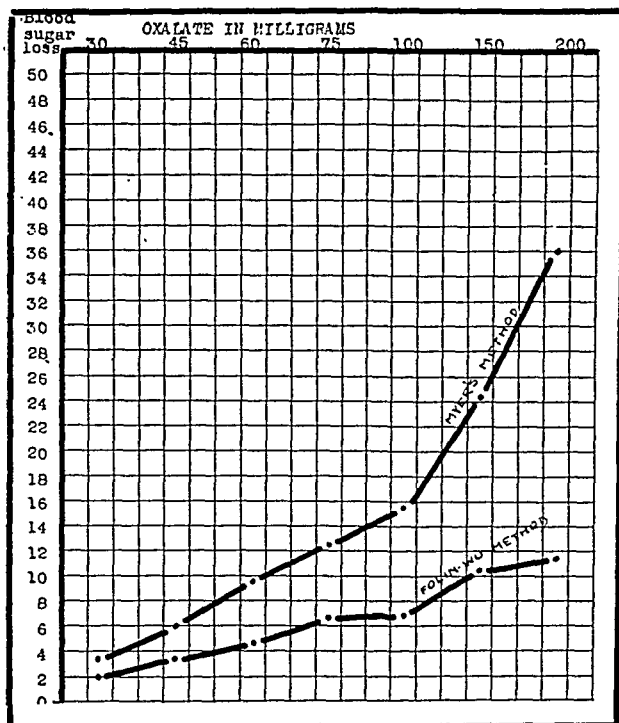
Chart 2.—Percentile decrease in the blood sugar content produced by varying amounts of potassium oxalate. Diabetic cases.

The explanation of this effect of potassium oxalate on the blood sugar determination must be left to the physiologic chemist. As a clinician, my interest lies in its practical application to our everyday work. It follows that in order to secure uniform and accurate results, the amount of oxalate used must be standardized. The need for such a standardization is indicated also by the variation in the amounts recommended by different authors. Thus, in the United States Army Manual and by Mathews, 50 mg. of oxalate per 10 c.c. of blood is recommended, while Folin, Cummer and Stitt advise 20 mg.

Because of this discrepancy, I made an investigation to see how large a quantity of blood could be kept from clotting by 15 mg. of oxalate. I set up a series of test tubes, each containing 15 mg. of oxalate, and varied the amount of

TABLE IV

A. BLOOD SUGAR VALUES IN NONDIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Folin-Wu Method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
	15	30	45	60	75	100	150	200	
Case 1	139		139		131		131	121	Blood
2	116	111	110		110		108	101	sugar
3	108	100	95	95	91	91	91	66	mg./100
4	122		118		117		114	113	c.c.
5	106	105	103	103	103	98	97	92	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1		0		6		6	13	
2	4	5		5		7	13	
3	7	12	12	16	16	16	39	
4		3		4		7	7	
5	1	3	3	3	8	9	13	
Average Loss	4	4.6	7.5	6.8	12	9	17	Per cent

TABLE V

A. BLOOD SUGAR VALUES IN DIABETIC CASES OBTAINED BY USING VARYING PORTIONS OF POTASSIUM OXALATE AS AN ANTICOAGULANT

(Folin-Wu Method)

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
	15	30	45	60	75	100	150	200	
Case 1	325			323		323		286	Blood
2	175	165	158	152	152	152	148	142	sugar
3	351		339		339	328		323	mg./100
4	155	155	155	155	146	146	144	142	c.c.
5	182	182	182	174	174	172	166	166	

B. PERCENTILE DECREASE IN EACH BLOOD SUGAR ESTIMATION AS COMPARED WITH (1) IN A

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
Case 1			1		1		12	
2	6	10	13	13	13	15	19	
3		4		3	7		8	
4	0	0	0	6	6	7	9	
5	0	0	4	4	6	9	9	
Average Loss	2	3.25	4.5	6.5	6.6	10.33	11.4	Per cent

TABLE VI

AVERAGE PERCENTILE LOSS OF BLOOD SUGAR
(Due to varying amounts of Oxalate)

NONDIABETIC CASES

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200
Percentile loss Folin-Wu Method	4	4.6	7.5	6.8	12	9	17
Percentile loss Myers' Method	2.8	4.66	9.6	17	16.37	34	48

DIABETIC CASES

MG. OF POT. OXALATE PER 2 C.C. BLOOD	(2) 30	(3) 45	(4) 60	(5) 75	(6) 100	(7) 150	(8) 200
Percentile loss Folin-Wu Method	2	3.25	4.5	6.5	6.6	10.33	11.4
Percentile loss Myers' Method	3.25	6	9.5	12.5	15.5	24.3	36

TABLE VII

THE AMOUNT OF BLOOD WHICH 15 MG. OF POTASSIUM OXALATE WILL KEEP FROM CLOTTING

Case 1					
Pot. Oxalate mg.	15	15	15	15	15
Blood c.c.	2	4	6	8	10
Clotting	neg.	neg.	neg.	neg.	neg.
Blood Sugar mg./100 c.c.	90	90	90	90	90
Case 2					
Pot. Oxalate mg.	15	15	15	15	
Blood c.c.	1	3	8	15	
Clotting	neg.	neg.	neg.	clotted	
Blood sugar mg./100 c.c.	113	113	113	113	

blood put into each, with the results shown in Table VII which demonstrates that when 10 c.c. of blood or less is used, 15 mg. of oxalate is sufficient to prevent clotting. Since the variations in the blood sugar estimation are small when the amount of oxalate varies between 15 and 30 mg., it would seem that for routine work it would be sufficient to place 15 mg. of oxalate in a test tube and use this as a guide for the amount to be placed in each tube used. It would not be practical and, as I have shown, it is unnecessary, to weigh each 15 mg. portion accurately, as that is time-consuming, and the error occurring from the slight increase, is hardly worth consideration. It is the use of the considerably larger portions of oxalate which needs to be eliminated. If all physicians and clinicians will adopt such a uniform basis, our figures will be more accurate and can be used fairly for comparative studies. There are many small technical problems connected with the study of diabetes, and it is only by solving each of these that we can properly evaluate our findings.

A POSSIBLE SOURCE OF ERROR IN THE USE OF RUSSELL'S DOUBLE SUGAR MEDIUM*

By William P. Belk, M.D., and Margaret Duff, Bryn Mawr, Pa.

IT has been the practice in this laboratory, and we believe elsewhere, in searching feces and urine for typhoid bacilli, to fish characteristic colonies from Teague's, or Endo, plates directly to Russell's double sugar tubes. If the reaction on this medium was characteristic, especially if there was no production of gas, further identification was undertaken. Otherwise a negative result was reported.
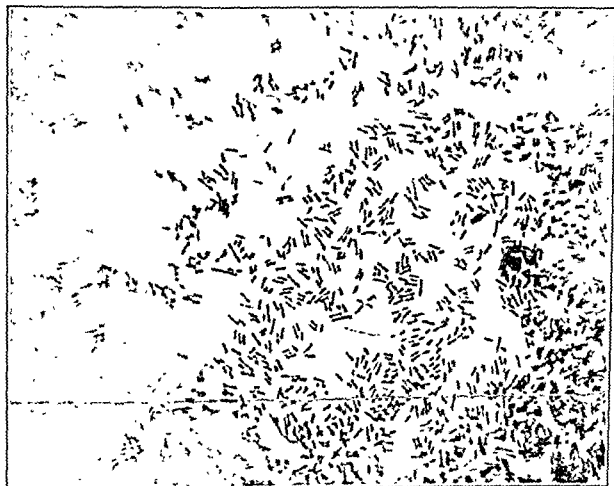
Fig. 1.—B. typhosus from Russell's double sugar medium showing vacuoles

Before making subcultures, or agglutination tests, Gram's stains were naturally studied from the growth on Russell's slants to determine purity of culture and typical morphology. It was in this step that our error occurred. It was found that typhoid bacilli, as well as other members of the Typhoid-Colon-Dysentery group, appeared, under these conditions, as large bacilli with vacuolated ends. The appearance was quite closely that of a spore-forming organism. Moreover, when litmus was the indicator in the Russell's medium,

ordinary decolorization in some instances left a bluish tinge in the bacteria, which gave them a Gram-positive appearance. This undoubtedly resulted in discarding cultures which merited complete identification.

This reaction was further studied with the following organisms: A stock strain of B. typhosus (U. S. Army); three cultures of B. typhosus from feces of a typhoid patient, identified by the specific agglutination; two strains of B. fecalis alcaligenes, and B. dysenteriae, B. Shiga (Stock). These were inoculated into the following sugar media; Russell's double sugar with litmus indicator; Russell's double sugar with Audrade's indicator; Teague's plates; and bouillon containing 1 per cent of glucose, lactose, saccharose, xylose, arabinose, mannite, maltose, dulcite, salicin, and inulin. All of the nine organisms showed well marked vacuolization on both Russell's tubes, and on Teague's plates. The stock strain of B. typhosus was vacuolated in every variety of sugar media. The other organisms were studied from the glucose and lactose bouillon only. Here the reaction was variable, some organisms showing many vacuoles, others none. This variation did not correspond at all to fermentation of the different sugars by the several bacteria; B. fecalis alcaligenes, for instance, showing a marked reaction in glucose, but none in lactose. With every series a plain agar slant was carried from which all the bacteria were typical morphologically.

Unstained bacteria, when treated with weak acid and weak alkali developed no color changes either in their bodies, or in the vacuoles, which would seem to eliminate vital staining of the organisms and the presence of the indicator in the vacuoles. Iodine likewise failed to produce any reaction. However, the definite association of this vacuolization with sugar media makes it seem reasonable that the carbohydrates influence this formation.

Incidentally it may be remarked that the acid reaction on Russell's medium was atypical in some respect in about 50 per cent of inoculations.

Conclusion: B. typhosus, and some other members of the Gram-negative intestinal group of bacilli, develop an atypical morphology on Russell's double sugar medium, being large and vacuolated. This medium is not a suitable one from which to study morphology.

LABORATORY DIAGNOSIS IN THROAT INFECTIONS[*]

II. The Direct Smear as an Aid to Diagnosis

By C. C. Young, Dr. P. H., and Minna Crooks, Lansing, Michigan

As indicated in a previous communication,[1] the routine use of the swab for transporting throat specimens permitted the preparation of a direct smear for immediate examination. We have found this procedure valuable in making a differential diagnosis and in saving time, as illustrated in Table I.

TABLE I
Direct Microscopic Examinations on Diagnostic Swabs
1923

CLINICAL DIAGNOSIS		LABORATORY FINDINGS	
	Direct Smear		Serum Media
	K. L.	V. A.	K. L.
Diphtheria	148	102	14	134
Septic sore throat	36	20	10	26
Vincent's angina	10	2	8	2
Tonsillitis	43	11	4	39
Doubtful	74	20	22	52
	311	155	58	253

K.L.—Klebs-Loeffler bacilli. V.A.—Organisms of Vincent.
(A comparison of the laboratory and clinical findings on positive diagnoses of three months in 1923.)

In Table I it may be observed that 155 swabs showed diphtheria bacilli and 58 showed organism of Vincent's on direct smear. Of 263 swabs found positive on culture, 155 (over 61 per cent) were found positive on direct examination. The early differential diagnosis made it possible to establish quarantine and immunize contacts eighteen to twenty-four hours earlier than otherwise in an appreciable number of cases.

The time factor is important in estimating the value of the direct microscopic examination in throat infections, but of much greater importance is the diagnosis of Vincent's angina, for it may be mistaken for diphtheria, as pointed out by Reckord and Baker[2] in a study of 56 cases. The incidence of Vincent's angina has no doubt been raised by the returned soldiers but on account of the lack of differential diagnosis, cases are missed. Organisms of Vincent were found on 5.7 per cent of the diagnostic swabs in the laboratories of the Michigan Department of Health during the fiscal year ending June 30, 1923, and were found in many cases which were clinically confused with diphtheria, as shown in Table I.

STATUS OF THE DIRECT MICROSCOPIC EXAMINATION

Since 1884, when Loeffler's cultural findings superseded Kleb's observations on morphology, the value of the direct smear in diphtheria diagnosis has

been questioned. Many authorities of today do not discuss it and those who do, recommend it with reservations. Park[3] recommends its use by experienced bacteriologists and bases a positive finding on contour, mode of division, and arrangement. Stitt[4] states that an immediate diagnosis is possible in 36 per cent of the cases and recommends Gram's method of staining. Osler in his textbook *"Practise of Medicine"* recommends an examination of a cover-glass preparation from membrane by an experienced bacteriologist as a confirmation of clinical diagnosis and the means of differentiating between pseudo and true membranes. He does not, however, advise its use by clinicians. Novy[5] men-
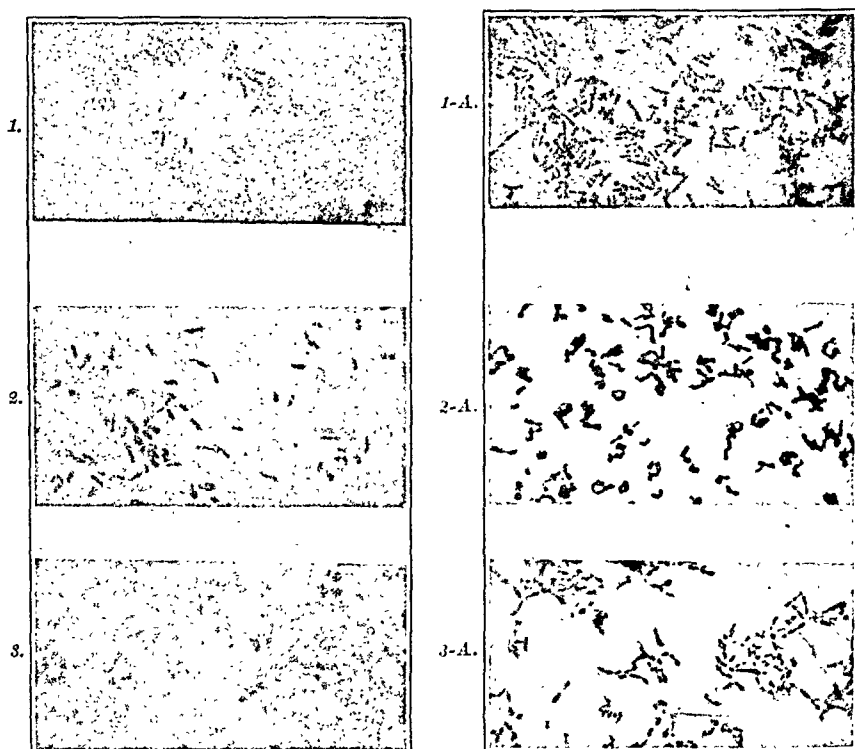
Plate 1.—Diphtheria bacilli in direct smears and in serum media. *1, 2, 3.* Direct slide preparations stained with alkaline methylene-blue. *1-A, 2-A, 3-A.* Smears of cultures from the swabs *1* and *3* respectively. *2.* Direct slide preparation from swab of sore on cheek stained with alkaline methylene-blue. *2-A.* Smear of culture of swab 2 stained by Gram's method. All cultures were pathogenic for guinea pig.

tions the cover-glass preparation from the membrane stained with methylene-blue as a method of procedure but does not recommend it.

Bleyer[6] discusses direct smears in diphtheria from the point of view of the clinician. In his investigation he was able to demonstrate diphtheria bacilli in 59 per cent of direct microscopic examinations made on exudates collected with a special "scoop" from known cases of diphtheria. He cites the frequent finding of Vincent's angina coexistent with diphtheria. Bleyer uses Greenthal's method of staining and recommends the direct smear as an aid to diagnosis if the clinician "seeks those forms with which he is familiar and trusts his judgment to no others."

SIGNIFICANCE OF MICROSCOPIC FINDINGS

The variability in the morphology of diphtheria organisms is an established fact. Westbrook, in 1900, classified diphtheria-like organisms morphologically and designated nineteen different types. He divided them into virulent and nonvirulent based upon his findings which showed that certain types were constant in diphtheria. Albert,[7] in 1920, used a differential staining method and classified them by their staining reaction into eight types. He found that cultures showing no granular forms were nonvirulent but did not find that all cultures showing granules were virulent. Laybourn, in 1921,[8] found morphology to be affected by the P$_H$ value of the medium and Albert[9] in the same year re-
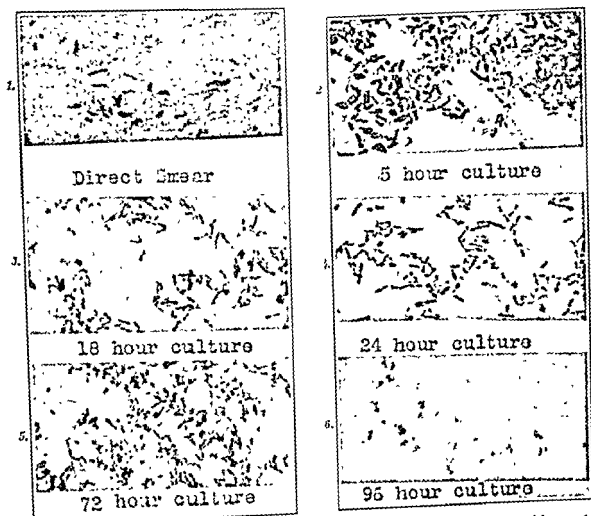
Plate 2.—Diphtheria bacilli stained with Loeffler's alkaline methylene-blue. *1.* Stained preparation from swab. *2, 3, 4, 5, 6.* Stained preparation from culture at different periods of incubation.

ports variations attributable to age of culture. Our experience with cultures on serum media mailed into the laboratory for virulence test has demonstrated the latter fact. Cultures on Loeffler's serum medium showing no typical bipolar staining organisms on arrival induced toxemia and death in guinea pigs when inoculated by the whole culture method. Stovall, in 1923,[10] studied the influence upon morphology and staining of B. diphtheria by growth in mixed cultures and found that the change was so marked as to completely alter the classification of the diphtheria bacilli when Westbrook's classification was used. It would seem from this brief survey that the recognized variations in the morphology of diphtheria bacilli in the stained slide preparations from throat swabs are attributable to (1) reaction and constituents of the medium supplied

by the mucous membrane of the throat; (2) growth in symbiosis with other organisms; and (3) age of organisms. Consequently, we observe on the direct smear the solid type and the clubbing distal ends of the young cultures (Plates 1 and 2), the metachromatic granules of the routine culture, or the pleomorphism of the old culture when the throat furnishes optimum conditions of growth, depending on the period of incubation, temperature, and P_H of the pabulum.

Freshly collected material from the throat no doubt has the greatest diagnostic value. Swabs one to three days in the mails, however, routinely give in these laboratories positive findings in both diphtheria and Vincent's angina. The procedure is in brief as follows:
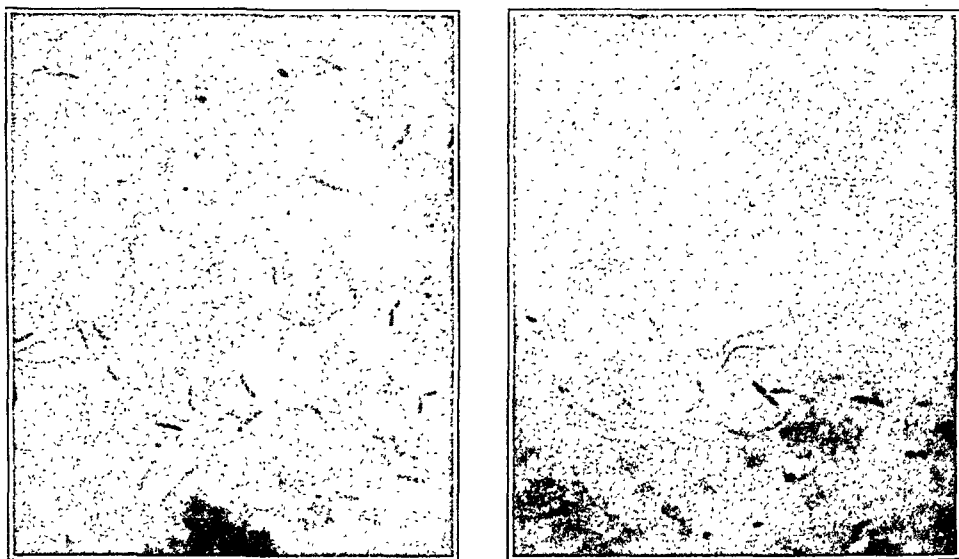
Plate 3.—Photographs of slide preparations from throat swabs stained with alkaline methylene-blue showing organisms of Vincent.

*1. First, inoculate blood plate; second, inoculate Loeffler's serum; make smear on slide.

NOTE: Only when freshly collected specimens are submitted are the slide preparations made before inoculating the culture mediums.

2. Examination of direct slide preparations.
   a. Stain with Loeffler's methylene-blue.
      To stain: Dry and fix preparation. Flood slide with stain for two minutes, wash in water, dry in air.
   b. Report only positive findings on direct smears. If a report is requested, give only a provisional negative report and make a final report from the culture.
   c. Report organisms of Vincent when B. fusiformis is found in the presence of spirocheta. (See Plate 3.)
3. General Ruling.
   a. At 8:00 A.M. daily of after ten to 18 hours incubation, spread smears, stain with Loeffler's alkaline methylene-blue and examine.
   b. Any cultures which show solid forms (A2 and C2) or overgrowth of other organisms should be smeared again and stained by a differential stain to demonstrate granules. Cultures showing granules and doubtful morphology should be stained by Gram's method.

*From the direction sheet of the Bureau of Laboratories, Michigan Department of Health.

We routinely use alkaline methylene-blue for two reasons: (1) the various differential stains do not stain the young culture which is one of the principle diagnostic features of the microscopic picture in direct smears; (2) we do not feel that the experienced microscopist needs differential stain in order to identify diphtheria bacilli. For, since we make our identification of diphtheria bacilli upon characteristic morphology, arrangement and bipolar staining, we want a stain that will give us a clear-cut picture of arrangement and morphology.

SUMMARY

A report of the findings on direct examinations of throat swabs is presented. Smears made from swabs mailed into the laboratory showed diphtheria-like bacilli on about 60 per cent of the positive cultures. The organisms of Vincent were found in many cases which were clinically confused with diphtheria. Smears from both the swabs and cultures were stained with Loeffler's alkaline methylene-blue and a diagnosis made upon staining reaction, morphology, and arrangement. Microphotographs are submitted, showing the effect of incubation period on morphology and the similarity of the microscopic picture in direct smear and in the young culture.

REFERENCES

[1]Young, and Crooks: Laboratory Diagnosis in Throat Infections. I. The Blood Plate as an Aid to Diagnosis, JOUR. LAB. AND CLIN. MED., March, 1924, ix, 409.
[2]Reckord, and Baker: Vincent's Angina Infection, Jour. Am. Med. Assn., Dec. 11, 1920, lxxiv, 1620.
[3]Park, et al.: Pathogenic Microorganisms, Philadelphia, 1920, Lea and Febiger, p. 351.
[4]Stitt: Practical Bacteriology, Blood Work and Animal Parasitology, Philadelphia, 1923, P. Blakiston's Son and Co., p. 151.
[5]Novy: Laboratory Work in Bacteriology, 1899, p. 334.
[6]Bleyer: Concerning Direct Smears in Diphtheria, Am. Jour. Dis. Child. 1920, xx, 445.
[7]Albert: A Classification of Diphtheria Bacilli Based on the Toludin Blue Iodine Method of Staining, Am. Jour. Pub. Health, December, 1920, x, 936.
[8]Laybourn: The Effect of the Reaction of Media Upon the Morphology of the Diphtheria Bacillus, Author's Abstract. Abstracts of Bact., 1921, v, 14.
[9]Albert: Variations in Morphology of the Diphtheria Bacillus Due to Age, Author's Abstract, Abstracts of Bact., 1921, v, 14.
[10]Stovall, et al.: The Influence of the Morphology and Staining of B. Diphtheria by Growth in Mixed Cultures, Am. Jour. Pub. Health, September, 1923 xiii, 748.

CONTRIBUTING CAUSE OF TURBIDITY OF NESSLERIZED SOLUTIONS IN THE DETERMINATION OF UREA IN THE WHOLE BLOOD*

BY FRANK B. COOPER, TRUDEAU, N. Y.

IN the determination of urea in blood during the past few years, we have noticed along with other observers, that turbidity frequently developed after nesslerization of the unknown solution.

The technic employed is that described by Gradwohl and Blaivas[1] and offers the advantages of speed, accuracy and ease of manipulation. This method is practically the same as that of Van Slyke and Cullen[2] except that the final ammonia solution instead of being titrated, is nesslerized as in Folin and Farmer's[3] method for total nitrogen in urine.

It was found, however, that a certain number of determinations failed, on account of the development of turbidity shortly after addition of Nessler's reagent to the final ammonia solutions. In no instance did the standards give turbid solutions.

After carefully checking over the various reagents used, the cause was traced to the small amount of caprylic alcohol, added to prevent foaming, which was carried over with the ammonia during aeration. The degree of turbidity produced varies directly, and the time of turbidity inversely with the amount of alcohol used.

Fresh samples of caprylic alcohol manufactured by three different companies were next tried and yielded practically identical results.

It was found that 0.02 c.c. of caprylic alcohol prevented foaming for twenty-five minutes, and gave solutions which remained clear for eight hours after addition of Nessler's reagent. Four-hundredths cubic centimeter prevented foaming for forty minutes and gave solutions remaining clear for one-half hour. Amounts over this prevented foaming for longer periods, but caused turbidity in ten to fifteen minutes.

Various antifoaming agents were next tried, some of which gave even greater turbidity than caprylic alcohol. The best was found to be a mixture of 1 c.c. of toluol and 0.02 c.c. of caprylic alcohol. This mixture prevented foaming for at least forty-five minutes, ample time for the aeration, and gave nesslerized solutions which remained clear for at least one hour.

REFERENCES

[1] Gradwohl, R. B. H., and Blaivas, A. J.: The Newer Methods of Blood and Urine Chemistry, St. Louis, 1917, C. V. Mosby Co.

[2] Van Slyke, D. D., and Cullen, G. E.: A Permanent Preparation of Urease, and Its Use for Rapid Determination of Urea, Jour. Am. Med. Assn., May 16, 1914, lxii, 1558-1559.

[3] Folin, O., and Farmer, C. J.: A New Method for the Determination of Total Nitrogen in Urine, Jour. Biol. Chem., April 12, 1912, xi, 493-501.

CLINICAL STUDIES ON THE KAHN REACTION FOR SYPHILIS[*]

II. SPECIFICITY OF TEST

By Harther L. Keim, M.D., Ann Arbor, Mich. and R. L. Kahn, Sc.D.,
Lansing, Mich.

A TEST for syphilis should conform with two major requirements. It should possess a high degree of sensitiveness in detecting the presence of syphilis and it should possess specificity, so that a positive reaction will be indicative of no pathologic condition except syphilis

The sensitiveness of the Kahn precipitation test has been considered in the first paper of this series.[1] The test was compared with two separate Wassermann systems, one of conservative sensitiveness (one hour fixation) and one of high sensitiveness (overnight fixation). The cases of syphilis studied totaled 625. These included: primary, 25; secondary, 64; tertiary, excluding cerebrospinal, 87; cerebrospinal, 192; latent, 218; congenital, 39. It was observed that the diagnostic value of the Kahn test approximated in its results the highly sensitive Wassermann test rather than the one of conservative sensitiveness.

This study was undertaken to find to what degree the Kahn test is specific for syphilis alone. We, herein, present the results of examinations of 2,500 nonsyphilitic cases at the University of Michigan Hospital.

THE KAHN TEST IN NONSYPHILITIC CASES

As in the first of this series of studies, the blood from each case was examined with the Kahn precipitation test and with two separate Wassermann tests. In each of these two tests a cholesterinized antigen was employed with ice-box fixation; one using an eighteen hour and the other a one hour fixation period. In the case of the Kahn test, no incubation was employed, the results being read immediately after mixing the ingredients.[2, 3]

Of the 2,500 cases studied, negative results with all three methods were obtained in 2,490 cases. These cases include: dermatologic, 750; medical, 495; surgical, 498; gynecologic, 151; otologic, 183; ophthalmologic, 235; and neurologic, 178. A classification of these cases is given in Table I. Positive reactions with one or more of the methods were obtained in the remaining ten cases. Of this number, the Kahn test gave seven reactions varying from one to three plus, representing an error of twenty-eight hundredths of one per cent. The findings are summarized in Table II.

DISCUSSION OF RESULTS

Of special interest in these results is the finding that the Kahn test gave no false reactions in the febrile diseases, including pneumonia and scarlet

TABLE I

CLASSIFICATION OF 2,490 NONSYPHILITIC CASES IN WHICH THE KAHN TEST AND TWO SEPARATE
WASSERMANN TESTS GAVE NEGATIVE REACTIONS

GROUP	DIAGNOSIS	NO. OF CASES
DERMATOLOGY	Acne (various types)	82
	Dermatomycosis	76
	Verruca	71
	Epithelioma	42
	Scabies	39
	Seborrheic dermatitis	35
	Psoriasis	33
	Herpes	29
	Dermatitis venenata	25
	Eczema	24
	Cutaneous tuberculosis	19
	Pityriasis rosea	18
	Furunculosis	18
	Chancroid	16
	Urticaria	16
	Impetigo contagiosa	15
	Erythema multiforme	13
	Scarlet fever	11
	Measles	6
	Varicella	5
	Variola	3
	Miscellaneous	154
	Total	750
MEDICINE	Gastric disease (excluding neoplasm)	69
	Pulmonary tuberculosis	59
	Arthritis	51
	Cardiac disease (nonspecific)	43
	Diabetes mellitus	41
	Hyperthyroidism	33
	Nephritis	28
	Simple adenoma of thyroid	25
	Cholecystitis	19
	Constipation	16
	Bronchitis	12
	Pleurisy	10
	Asthma	9
	Pneumonia	6
	Miscellaneous	74
	Total	495
GYNECOLOGY	Salpingitis	26
	Carcinoma-uterus	17
	Vaginitis	12
	Sterility	14
	Retroversion-uterus	11
	Fibroid-uterus	10
	Prolapse-uterus	8
	Ovarian cyst	6
	Dysmenorrhea	5
	Miscellaneous	42
	Total	151

TABLE I—CONT'D

GROUP	DIAGNOSIS	NO. OF CASES
	Gonorrhea and complications	52
	Carcinoma gastrointestinal tract	51
	Injury	48
	Hernia	38
	Appendicitis	37
	Bone and joint tuberculosis	31
	Osteomyelitis	22
SURGERY	Prostatism	19
	Cholelithiasis	19
	Carcinoma breast	17
	Hemorrhoids	12
	Nephrolithiasis	11
	Carcinoma genitourinary tract	9
	Tuberculosis genitourinary tract	8
	Miscellaneous	124
	Total	498
	Refractive *error*	57
	Cataract	41
	Conjunctivitis	23
OTOLOGY	*Corneal ulcer*	9
	Glaucoma	5
	Trachoma	4
	Miscellaneous	44
	Total	183
	Tonsillitis	69
	Otitis media	26
	Sinusitis	25
	Rhinitis	21
OPHTHALMOLOGY	Nasal obstruction	19
	Mastoiditis	14
	Pharyngitis	9
	Tuberculous laryngitis	5
	Miscellaneous	47
	Total	235
	Hysteria	29
	Epilepsy	18
	Migraine	11
	Anxiety neurosis	10
	Multiple sclerosis	10
	Tic *douloureux*	8
NEUROLOGY	Chorea	7
	Manic depressive insanity	7
	Polyneuritis	6
	Dementia precox	6
	Brain tumor	5
	Paralysis agitans	4
	Miscellaneous	57
	Total	178

TABLE II

CLASSIFICATION OF CASES GIVING POSITIVE REACTIONS WITH ONE OR MORE METHODS

| NUMBER OF CASES | KAHN REACTION | WASSERMANN REACTION CHOLESTERINIZED ANTIGEN | | DIAGNOSIS |
		18 HOUR FIX- ATION ICE BOX	1 HOUR FIX- ATION ICE BOX	
1	+++	−	++	Pompholyx
1	++	−	+	Acne rosacea
1	+	−	±	Alopecia areata
1	+	±	±	Acne vulgaris
1	+	−	−	Acne vulgaris
1	+	±	−	Pityriasis rosea
1	++	++	−	Dermatomycosis
1	−	±	−	Pneumonia
1	−	++++	−	Deviated nasal septum
1	−	++++	−	Tonsillitis

fever, and that it gave no false reactions in diabetes, carcinoma and tuberculosis—pathologic conditions in which false Wassermann reactions are still reported, although infrequently.

The several positive reactions obtained are of additional interest. It is perhaps fair to state that the study herein reported extended throughout 1923 and up to July, 1924, and with the exception of two positive reactions (pityriasis rosea and dermatomycosis) all positives were obtained during 1923 before the Kahn test had undergone standardization. These reactions, in our opinion, illustrate that no biologic test can be error proof and emphasize the importance of interpreting the results of serologic tests only in the light of clinical evidence or history.

SUMMARY

A series of 2,500 cases, clinically free from syphilis, were studied with the Kahn precipitation test.

Negative reactions were obtained in 2,493 cases; one plus reactions were obtained in 4 cases; two plus reactions in 2 cases; and a three plus reaction in 1 case.

These results indicate that the Kahn test possesses a high degree of specificity for syphilis.

REFERENCES

[1]Keim, H. L., and Kahn, R. L.: Studies on the Kahn Reaction for Syphilis, I. Diagnostic Value of Test, Arch. f. Dermat. and Syph., December, 1924, x, 732.
[2]Kahn, R. L.: The Kahn Precipitation Test for Syphilis, Demonstration, Am. Jour. Pub. Health, June, 1924, xiv, 498.
[2]Kahn, .R. L.: Serum Diagnosis of Syphilis by Precipitation—Governing Principles, Procedure and Clinical Application of the Kahn Precipitation Test; Williams and Wilkins Co., Baltimore, 1925.

The following are some current references on the Kahn Test.

Rothberg, Wm.: A Comparative Study of the Kahn Precipitation Test with the Kolmer Modification of the Wassermann, Abst. Bacter., January, 1924, viii, 29.
Elliott, J. A., and Todd, L. C.: The Value of the Kahn Precipitation Test in the Diagnosis of Primary Syphilis and Its Adaptation to Local Fluids, Arch. f. Dermat. and Syph., February, 1924, ix, 208.
Malcolm, M.: Comparison of Kahn Test with Wassermann Test, Can. Med. Assn. Jour., March, 1924, xiv, 222.
Osmond, T. E., and McLean, D.: A Comparison of the Kahn and Wassermann Test in Five Hundred Serums, Brit. Med. Jour., April 5, 1924, 3301:617.

Rubinstein, M., and Gauran: Procéde de flocculation: Technique de Kahn, Bull. Soc. franc. de dermat. et de Syph., April, 1924, xxxi, 218.

Havens, L. C., and Taylor, M. L.: The Value of the Precipitation Test (Kahn) for Syphilis as a Public Health Laboratory Procedure, Am. Jour. Pub. Health, April, 1924, xiv, 323.

Anderson, J. A., and Fisher, E. B.: A Further Report on the Kahn and Wassermann Tests of Blood Serum and Spinal Fluid, Jour. N. J. Med. Sc., April, 1924, xxi, 132.

Keim, H. L.: Further Observations of the Kahn Reaction in the Serologic Diagnosis of Syphilis, Am. Jour. Syph., April, 1924, viii, 323.

Podesta, A.: La nueva reacción de precipitación rápida de Kahn para la sifilis, Semana med., May 1, 1924, i, 827.

Ishii, O.: Comparative Studies on the Kahn Precipitation Test for Syphilitic Serum and the Wassermann Test, Arch. f. Dermat. u. Syph., May, 1924, ix, 612.

Rockstraw, E. W., and Hopkins, J. G.: Zone Phenomena in the Kahn Precipitation Test for Syphilis, Proc. Soc. Exper. Biol. and Med., May, 1924, xxi, 453.

Young, C. C.: The Kahn Precipitation Test for Syphilis: Two Years' Experience in a Public Health Laboratory, Am. Jour. Pub. Health, June, 1924, xiv, 508.

Rockstraw, E. W., and Bent, M. J.: A Comparison of the Wassermann, Sachs-Georgi and Kahn Tests for Syphilis, JOUR. LAB. AND CLIN. MED., June, 1924, ix, 624.

Wilson, M. A., and Nedley, R. M.: Comparison of Wassermann and Precipitation Reactions in Various Stages of Syphilis, JOUR. LAB. AND CLIN. MED., July, 1924, ix, 704.

Franklin, G. O. H.: Kahn Precipitation Reaction for Syphilis, Mil. Surgeon, July, 1924, lv, 39.

Kendrick, P. L.: Effect of Contamination and Age of Serum on Kahn Precipitation Test, Am. Jour. Pub. Health, August, 1924, xiv, 673.

Dudgeon, J. S.: The Kahn Test in General Paralysis, The Lancet, London, September 20, 1924, ii, 599.

Kilduffe, R. A.: Incidence of the Kahn Flocculation Reaction in Tuberculosis, Med. Jour. and Record, July, 1924, cxx, supplement p. xiii.

Johnson, F. B., and Wellbrock, W. L. A.: Wassermann and Kahn Reactions, Southern Medicine and Surgery, April, 1924; see Med. Jour. and Record, September, 1924, cxx, 304.

Blackshear, E.: The Kahn Precipitation Test Compared with the Wassermann, Mil. Surgeon, November, 1924, lv, 662.

Levin, W.: The Kahn Precipitation Test as a Routine Test in the Diagnosis and Treatment of Syphilis, Northwest Medicine, December, 1924, xxiii, 543.

Sheppe, W. M., and Rogers, I. C.: Kahn Precipitation Reaction in Diagnosis of Syphilis, West Va. Med. Jour., December, 1924, xix, 639.

Biernacki, W., and Landau, J.: Odczyn Kilowy Kahna, Polskiej Gazety Lekarskiej, 1924, xli.

Monthly Bull. Department of Health, City of New York, Bureau of Laboratories, 1924, xiv, 224 (Kahn Test: Two Studies)

Masuda, R.: On Kahn's and Sachs-Georgi's Reactions, Japan Dermat. Urol. Assoc. Syphil. Sect. Niigata 26, a, 27, iii, 1924; see Zentralbl. für Haut und Geschlechts Krankheiten, February, 1925, xvi, 245.

Parham, J., and Behrens, C. F.: The Kahn Precipitation Test as Performed Aboard the U.S.S. "Henderson," U. S. Nav. Med Bull., January, 1925, xxi, 23.

Owen, R. G., and Cope, H. E.: The Kahn Precipitation Test for Syphilis, Jour. Mich. State Med. Soc., February, 1925, xxiv, 94.

HEMOGLOBIN AND IRON IN BLOOD*

By Guy E. Sackett, B.S., M.S., Kansas City, Mo.

THE estimation of hemoglobin is a very unsatisfactory procedure because of the lack of a uniform standard in common for all methods of determining hemoglobin. Most hemoglobinometers are graduated to read in percentage, considering 100 per cent as the normal standard; but yet, each has a different number of grams of hemoglobin which is supposed to correspond to 100 per cent.

Haldane[1] has considered the oxygen capacity of the blood of normal persons to be 18.5 c.c., corresponding to 13.8 grams of hemoglobin per 100 c.c. If the grams of hemoglobin in the blood of normal individuals are determined by the oxygen capacity method or by the iron method and expressed in percentage considering 13.8 grams as 100 per cent, the resulting percentage of hemoglobin will be about 115 per cent, which is, of course, misleading.

Haden[2] working with the Van Slyke oxygen capacity method, found the normal standards to be 15.6 grams of hemoglobin per 100 c.c. of blood.

TABLE I

HEMOGLOBIN AND IRON IN BLOOD OF APPARENTLY NORMAL MEN

NAME	RED CELL COUNT	HEMOGLOBIN NEWCOMER METHOD	IRON (FE) MG. PER 100 C.C.	HEMOGLOBIN GM. PER 100 C.C. CALCULATED FROM IRON	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO 100% HEMOGLOBIN BY NEWCOMER METHOD	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO A RED CELL COUNT OF 5 MILLION
W. A. M.	4,896,000	98.5%	55.5	16.56	16.81	16.91
C. W. E.		95.9	53.5	15.97	16.65	
H. M. C.	4,872,000	99.1	55.5	16.56	16.71	16.99
J. D. L.	5,216,000	102.7	57.4	17.13	16.67	16.51
F. L.	4,896,000	98.0	55.5	16.56	16.89	16.91
J. L. H.	5,344,000	101.3	56.0	16.71	16.49	15.63
C. N. C.	4,882,000	99.7	56.4	16.82	16.87	17.22
J. A.	5,216,000	101.3	57.0	17.01	16.79	16.30
J. H. H.	4,832,000	100.0	55.6	16.59	16.59	17.16
H. W.	5,920,000	116.0	63.3	18.89	16.19	15.95
T. J. D.	5,760,000	114.1	64.7	19.30	16.91	16.75
E. M.	4,704,000	94.6	50.2	14.98	15.83	15.92
F. L. S.	4,804,000	95.9	51.5	15.37	16.02	15.99
F. C.	4,800,000	98.4	52.6	15.70	15.95	16.35
D. E. P.	5,160,000	101.0	55.6	16.59	16.42	16.07
Average	5,093,000	101.1	56.02	16.71	16.52	16.47

Average Color Index 0.9925

Newcomer[3] has recently taken 16.92 grams of hemoglobin per 100 c.c. of whole blood, the average obtained by Williamson[4] for adult males, as his standard of 100.

The object of this work is to compare the percentage of hemoglobin found by Newcomer's method with the grams of hemoglobin present, and also to help establish a normal hemoglobin standard expressed in grams per 100 c.c. corresponding to a five million red cell count.

The grams of hemoglobin are calculated from the iron content of the blood. This method used for determining the iron is that of San Yin Wong.[5] To obtain grams of hemoglobin, the number of milligrams of iron per 100 c.c. of blood is divided by 3.35 (since hemoglobin contains 0.335 per cent of iron). The pres-

TABLE II

HEMOGLOBIN AND IRON IN BLOOD OF APPARENTLY NORMAL WOMEN

NAME	RED CELL COUNT	HEMOGLOBIN NEWCOMER METHOD	IRON (FE) MG. PER 100 C.C.	HEMOGLOBIN GM. PER 100 C.C. CALCULATED FROM IRON	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO 100% HEMOGLOBIN BY NEWCOMER METHOD	GM. OF HEMOGLOBIN PER 100 C.C. CORRESPONDING TO A RED CELL COUNT OF 5 MILLION
P. M.	4,640,000	93.4%	50.8	15.16	16.23	16.33
M. T.	4,800,000	97.0	54.0	16.12	16.62	16.79
B. H.	4,636,000	95.9	51.8	15.46	16.12	16.67
M. A.	4,384,000	88.7	45.7	13.64	15.37	15.55
E. R.	4,800,000	94.6	51.5	15.37	16.24	16.00
F. B.	4,480,000	87.7	47.6	14.21	16.20	15.86
C. E.	5,152,000	100.0	54.6	16.30	16.30	15.82
A. C.	4,289,000	88.7	47.6	14.21	16.02	16.57
J. E.	4,192,000	83.6	45.9	13.70	16.38	16.34
S. C.	4,160,000	84.6	46.3	13.79	16.30	16.57
M. S.	5,312,000	102.7	57.5	17.16	16.71	16.16
K. R.	5,180,000	100.3	55.6	16.59	16.54	16.01
A. S.	4,640,000	96.0	53 7	16.03	16.69	17.27
E. B.	4,800,000	97.1	54.3	16 21	16 69	16.88
Average	4,676,000	93.59	51.20	15.28	16.32	16.34

Average Color Index 1.0008

ence of iron in the chemicals used was ruled out by blank determinations. The blood counting pipette, counting chamber, and cover glass used in this work were calibrated by comparison with apparatus from the U. S. Bureau of Standards.

CONCLUSIONS

1. One hundred per cent hemoglobin by Newcomer's method represents 16.4 grams of hemoglobin per 100 c.c. of whole blood.

2. The normal standard corresponding to a five million red cell count is 16.4 grams of hemoglobin per 100 c.c. of whole blood.

REFERENCES

[1]Haldane, J. S.:   The Colorimetric Determination of Hemoglobin, Jour. Physiol., 1900-01, 497-504.
[2]Haden, R. L.:   Normal Hemoglobin Standard, Jour. Am. Med. Assn., 1922, lxxix, 1496-1497.
[3]Newcomer, H. S.:   A New Optical Instrument for the Determination of Hemoglobin, Jour. Biol. Chem., 1923, lv, 569-574.
[4]Williamson, C. S.:   Influence of Age and Sex on Hemoglobin, A Spectrophotometric Analysis of Nine Hundred and Nineteen Cases, Arch. Int. Med., 1916, xviii, 505-528.
[5]Wong, S. Y.:   Colorimetric Determination of Iron and Hemoglobin in Blood, Jour. Biol. Chem., 1923, lv, 421-425.

COMPARATIVE HEAT RESISTANCE OF SERUMS IN THE KAHN AND WASSERMANN TESTS*

By Pearl L. Kendrick, B.S., Lansing, Mich.

IT is generally accepted that prolonged heating of syphilitic serums at 56° C. tends to weaken Wassermann reactions, the explanation being that at this temperature complement-fixing substances are destroyed.   According to Noguchi,[1] serums heated for thirty minutes at 56° C. may be 50 per cent less potent than unheated serums.   Simon[2] and Kolmer[3] also report the apparent destruction of complement-fixing substances at 56° C.   Simon recommends the heating of serums for ten minutes, and Kolmer for fifteen minutes, instead of the usual heating for thirty minutes.   In the case of the Kahn precipitation test, it was shown in this laboratory[4] not only that heating serums at 56° C. was essential for precipitation reactions but that heating beyond thirty minutes often gave stronger reactions.   This finding suggested a marked difference in the effect of heat on complement fixing and precipitin substances, the former showing apparent destructibility and the latter no destructibility at 56° C.

In the experiments to be reported in this paper an attempt was made to study the comparative effect on the Kahn and Wassermann tests of heating the same serum for various periods.   This, it was believed, would give a more correct picture of the relative behavior of the tests with heated serums than experiments in which the Wassermann test was employed with one group of serums and the Kahn test with another group.

EXPERIMENTAL

Syphilitic and nonsyphilitic serums were tested with the Wassermann and Kahn tests before heating and after heating for various periods.   The Wassermann test was carried out with a sheep cell—guinea pig complement system.   All ingredients entering into the test were used in 0.1 c.c. amounts except the serum which was employed in 0.01 and 0.02 c.c. amounts.   Two units of amboceptor, two units of complement and three units of a 0.4 per cent cholesterinized antigen were employed.   The fixation period was one hour at ice box temperature.   The Kahn precipitation test was carried out in

the usual manner, employing the standardized routine test. Because of the comparatively large number of examinations made on each specimen, only one tube was employed for each test—the one containing 0.025 c.c. antigen dilution and 0.15 c.c. serum.

Two distinct series of experiments were carried out—the first to determine the effect of heating serums at 56° C. for various periods and the second to determine the effect of heating serums at a higher temperature. In this second series, the temperature chosen was 62° C since the serum proteins are readily coagulated at still higher temperatures. A large number of the specimens used were diluted with physiologic salt solution to give a sufficient quantity for the several separate examinations. It may be mentioned here that the undiluted specimens showed the same general behavior to heat as the diluted ones and the results of all are, therefore, included together. In each series there was included a group of syphilitic serums and a group of nonsyphilitic serum controls—the classification being based upon the Wassermann and Kahn findings in the routine examinations in this laboratory.

The first series included 66 syphilitic and 36 nonsyphilitic serums. These specimens were examined before heating and after heating periods of thirty, ninety, one hundred and twenty, and one hundred and eighty minutes, respectively, at 56° C. The specimens were prepared for the tests as follows: Two-tenths c.c. was first removed from each specimen before heating. The specimens were then placed in the water-bath at 56° C. for thirty minutes, at the end of which period 0.2 c.c. was removed. The specimens were returned to the water-bath for the next heating period and so on until a portion of each specimen had been removed at the end of each heating period. During the heating periods, the small portions which had been removed were covered with a damp towel to prevent evaporation. When the five portions of each

TABLE I

Effect of Heating Serums at 56° C. on Wassermann and Kahn Tests

SERUM NO.	PERIODS OF HEATING AT 56° C									
	NONE		30 MIN.		90 MIN.		120 MIN.		180 MIN.	
	WASS.	KAHN.	WASS.	KAHN.	WASS.	KAHN	WASS.	KAHN.	WASS.	KAHN.
	RESULTS									
1	++++	++++	++++	+++	+++	+++	+++±	++++	++++	++++
2	.*Ac	++++	++++	+++±	+++±	+++	++++	+++±	+++±	+++
3	++	+++	++	++++	±	++++	—	++++	—	+++
4	+++	++	+++	+++	++	++++	++	++++	±	+++
5	++	++	++	++	+	+++	+	++++	—	+++
6	++++	+	++++	++++	++++	++++	±+++	+++±	+—++	+++
7	++	+	++	±	+	++	±	+++	—	++
8	++++	±	++++	+++±	++++	++++	±	+++±	++±	+++
9	++++	±	+±	+++	±	++++	±	++++	±	+++
10	+++	±	++	++++	±	++++	++	+++±	++	+++
11	++	±	++++	++++	±	++++	—	++++	—	+++
12	±	±	±	++	—	++++	—	++++	—	+++
13	+	±	++++	+++	++	++++	++	+++±	±	+++
14	Ac	—	—	+	+	++++	±	++++	—	+++
15	++++	—	++++	+++	++++	+++±	++	++++	+	+++
16	+++	—	+++	±	++	+	+	++	+	+++
17	++	—	+	+	+	++	±	+++	—	+++
18	++	—	++	+	+	+++±	±	+++	—	+++
19	++	—	±	++	—	++	±	+++	—	+++
20		+	+	++	—	++	±	+++		+++

*Ac=Anticomplementary.

specimen were ready for testing, they were examined by the Wassermann and Kahn tests. The negative control serums gave negative results throughout. Table I gives the findings with twenty of the positive serums. These results are typical of those given by the whole group.

Before heating, the serums almost invariably gave stronger reactions with the Wassermann test than with the Kahn test. After thirty minutes heating at 56° C., the reactions became stronger with the Kahn test and were comparable with those of the Wassermann. Beyond thirty minutes heating, the reactions with the Wassermann became gradually weaker while the reactions with the Kahn either remained the same or became stronger up to three hours heating. Only an occasional precipitation reaction became weaker even after three hours heating of the serum at 56° C.

The second series of experiments included 101 syphilitic and 21 nonsyphilitic serums. After the routine inactivation period of thirty minutes at 56° C., these specimens were heated for periods of thirty, sixty, and one hundred and twenty minutes, respectively, at 62° C. The several portions of each specimen were removed at the end of the various heating periods as described in the first series and then examined by Wassermann and Kahn tests. The negative serum controls gave negative results throughout. Table II shows the findings with twenty positive serums and, as in the first series, they are typical of the results obtained with the whole group.

TABLE II

EFFECT OF HEATING SERUMS AT 62° C. ON WASSERMANN AND KAHN TESTS

HEATING PERIOD	AT 56° C. FOR 30 MINUTES		AT 62° C. FOLLOWING 30 MINUTES AT 56° O.					
			30 MIN.		60 MIN.		120 MIN.	
SERUM NO.	WASS.	KAHN	WASS.	KAHN	WASS.	KAHN	WASS.	KAHN
	RESULTS							
1	++++	++++	++++	++++	++++	++++	++++	+++
2	++++	++++	++++	++++	++++	++++	*Ac	+++
3	++++	++++	+++	++++	++	++++	++	+++
4	++++	++++	+++	++++	+++	++++	−	++
5	++++	++++	+++	++++	++++	+	−	−
6	++++	++++	+++	++++	+	++++	±	+
7	++++	++++	Ac	+++	Ac	++	Ac	−
8	++++	++++	+	++++	±	++++	−	++
9	++++	++++	+++	++++	Ac	+	−	−
10	++++	++++	−	++++	−	++++	−	±
11	±	++++	−	+	−	−	−	−
12	++++	++++	+	+	−	−	−	−
13	+	+++	−	+++	−	+	−	−
14	++++	+++	±	+++	−	+++	−	++
15	++++	++	++	++	−	+	−	+
16	++	++	−	++	−	++	−	++
17	++++	++	++	++	−	+++	−	+++
18	+	+	−	−	−	−	−	−
19	−	+	−	+	−	±	−	−
20	++	±	++	+++	++	+++	−	++

*Ac=Anticomplementary.

After thirty minutes heating at 62° C., the Wassermann reactions were considerably weaker than after thirty minutes at 56° C. On the other hand, the Kahn reactions showed comparatively little change; a few reactions became somewhat weaker and a few slightly stronger. Beyond thirty minutes heating at 62° C. most of the Wassermann reactions became negative and the Kahn reactions weaker.

CONCLUSIONS

1. Unheated serums give stronger reactions in the Wassermann than in the Kahn test.

2. Serums heated for thirty minutes at 56° C. give comparable reactions in the Kahn and Wassermann tests.

3. After prolonged heating of serums at 56° C.—up to three hours—there is marked weakening of Wassermann reactions with a tendency towards stronger Kahn reactions.

4. Serums heated for thirty minutes at 62° C. show marked reduction in the strength of the Wassermann and comparatively lesser reduction in Kahn reactions.

5. The heating of serums beyond thirty minutes at 62° C. renders practically all Wassermann reactions negative and Kahn reactions weaker.

REFERENCES

[1]Noguchi: Serum Diagnosis of Syphilis, Second edition, Lippincott, p. 97.
[2]Simon: Jour. Am. Med. Assn., 1919, lxxii, 1534.
[3]Kolmer: Am. Jour. Syph., 1922, vi, 82.
[4]Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation-Governing Principles, Procedure and Clinical Application of the Kahn Precipitation Test, Baltimore, Williams and Wilkins Co., p. 82.

THE EFFECT OF SOME GUANIDINE DERIVATIVES AND OTHER RELATED SUBSTANCES UPON THE BLOOD SUGAR OF NORMAL RABBITS*

By Harry E. Dubin, Ph.D., and H. B. Corbitt, Ph.D., New York City

BASED upon the finding that guanidine sulphate produced a hypoglycemia in normal rabbits similar to that resulting after an injection of certain plant extracts, Collip[1] thought that insulin might possibly be a guanidine compound. He found that the hypoglycemia produced by plant extracts is characterized by the fact that low blood sugar does not develop until a number of hours after the injections. The return to normal is considerably delayed. With insulin, on the other hand, hypoglycemia develops in one or two hours and there is a return to normal in from four to six hours. Because of this difference, Collip believed that plant extracts contained a new hormone, which he named "Glucokinin."

Working with crude plant extracts we[2] obtained results exactly like Collip's. We explained the delayed blood-sugar reducing effect as being due to the presence of a blood-sugar increasing substance in crude extracts. When this substance was removed, it was no longer possible to obtain the delayed blood-sugar reducing effect. From the remaining extract, it was possible to isolate a substance which, when injected into normal rabbits, produced a fall in blood sugar exactly similar to that caused by insulin. Obviously therefore,

we could not support the theory of the presence of a new hormone in plant tissue.

In the case of guanidine sulphate, Collip[3] obtained the delayed blood-sugar decreasing effect, but the animals eventually died. It seemed plausible to assume that insulin might be a guanidine compound and with this in mind, we decided to investigate a number of derivatives and some related substances.

We have studied the effect of guanidine carbonate, methyl guanidine sulphate, guanidine nitrate, guanidine hydrochloride and guanidine thiocyanate. In addition, we tested histidine, present in germinating plants; xanthine, found in cellular tissue; and adenine, present in the pancreas. The experimental procedure has already been described.[2]

A study of the tables shows that histidine hydrochloride was without effect. Adenine sulphate caused a 10.4 per cent decrease in six hours, while xanthine produced a fall of 23.2 per cent in the same length of time. Observations for long periods of time, with these materials, were not made.

As for the various guanidine derivatives, the most pronounced hyperglycemia was obtained with methyl guanidine sulphate. The thiocyanate in one case caused a rise of 45.8 per cent in six hours. In another rabbit, there was an increase of 124.0 per cent in five and two-thirds hours. The animal died after twenty-five hours, the blood sugar at that time being 15.1 per cent below the original reading. This decrease is frequently observed as well in normal animals at death.

Of all the substances investigated, it may be said in general that small doses were ineffective. Larger doses did cause a reduction in blood sugar, but the animals eventually died. Our findings in this respect, therefore, confirm those of Collip.

At the same time, it should be noted that the decrease in blood sugar

TABLE I

BLOOD SUGAR OF NORMAL RABBITS AFTER INJECTION OF VARIOUS GUANIDINE DERIVATIVES

RABBIT		PREPARATION INJECTED	DOSE	TIME AFTER INJECTION	BLOOD SUGAR	CHANGE IN SUGAR CONTENT	REMARKS
NO.	WT.						
	GM.		MG. PER KG.	HR.	MG. PER 100 C.C.	PER CENT	
66	2105	Guanidine carbonate	10	0	112		
				2	115		
				4	111		
				6	111		
51	1570	Guanidine carbonate	50	0	135	+17.0	
				2	141		
				4	147		
				6	158		
55	1580	Guanidine carbonate	150	0	148		
				2	142		
				4	135		
				6	103	−30.4	
				23½	114		
				29	140		
56	1710	Guanidine carbonate	200	0	145		
				2	124		
				4	93		
				6	85	−43.4	Died at about 50 hours.
				24	98		
				29	85		

TABLE II

BLOOD SUGAR OF NORMAL RABBITS AFTER INJECTION OF VARIOUS GUANIDINE DERIVATIVES

RABBIT		PREPARATION INJECTED	DOSE	TIME AFTER INJECTION	BLOOD SUGAR	CHANGE IN SUGAR CONTENT	REMARKS
NO.	WT.						
	GM.		MG. PER KG.	HR.	MG. PER 100 C.C.	PER CENT	
49	920	Methyl guanidine sulphate	50	0	99		
				2¼	91		
				4½	86		Animal died
				6½	85	−14.1	25 hr.
68	1245	Methyl guanidine sulphate	100	0	95		
				2½	56	−41.0	
				3	—		Dead; bled
				3	25	−73.7	at once
59	1800	Guanidine nitrate	200	0	89		
				2	61		
				4	62		
				6	57		Died during
				0	138	−36.0	night
63	2150	Guanidine hydrochloride	180	2½	129		
				4	79		
				25	110	−42.7	
62	2245	Guanidine thiocyanate	180	0	126		
				2¼	196		Died at 25
				5¾	283		hr., bled
				25	107	+124.0	at once

TABLE III

BLOOD SUGAR OF NORMAL RABBITS AFTER INJECTION OF VARIOUS PURE COMPOUNDS

RABBIT		COMPOUND INJECTED	DOSE	TIME AFTER INJECTION	BLOOD SUGAR	CHANGE IN SUGAR CONTENT	REMARKS
NO.	WT.						
	GM.		MG. PER KG.	HR.	MG PER 100 C.C.	PER CENT	
46	3070	Histidine hydrochloride	100	0	104		
				2	107		
				4	102		
				6	116		
			500	0	119		
				2¼	117		
39	1610	Adenine sulphate	100	3½	131		
				4½	124		
				5½			
44	1365	Adenine sulphate	325	0	106		
				2	174	+64.2	
				4	187	+76.3	
				6	95	−10.4	
48	1740	Xanthine	50	0	95		
				2	88	− 7.4	Other animals gave
				4	84	−11.6	similar results.
				6	73	−23.2	

was not comparable, in any instance, with that produced by a unit of insulin. Accordingly, we feel that there is no relationship between insulin and any of the products tested.

REFERENCES

[1] Collip, J. B.: Jour. Biol. Chem., 1923, lvii, 65.
[2] Dubin, Harry E., and Corbitt, H. B.: Jour. Metab. Research, 1923, iv, 89.
[3] Collip, J. B.: Jour. Biol. Chem., 1923, lviii, 163.

THE VALUE OF EARLY WASSERMANN EXAMINATIONS IN THE DIAGNOSIS OF VENEREAL LESIONS*

BY ROBERT G. OWEN, A.M., M.D., AND H. E. COPE, B.S., M.D., DETROIT, MICHIGAN

IN May of 1923, this laboratory adopted the following routine for the diagnosis of suspected primary syphilitic lesions. At first examination, we obtain blood for a Wassermann test, in addition to the usual dark-field slides. Cases with negative dark-field and Wassermann examinations are recalled for future Wassermann examinations at stated intervals, to cover a period of six weeks from the onset of their lesion; i. e., the time within which a positive Wassermann usually develops.

In a survey of 1160 dark-field examinations we were astonished by the relatively large number of first examinations yielding negative dark-fields and positive Wassermanns. The consensus of opinion among those engaged in clinical laboratory work seems to be that a Wassermann is of little value if taken early in the course of a venereal sore, the onset of a positive Wassermann being generally recognized as occurring between the fourth and eighth week. In those cases in which we have followed the development of positivity, it has occurred quite uniformly between the twenty-first and thirty-sixth day from the appearance of the initial lesion.

In spite of the unreliability of the average venereal history, one cannot but be impressed when 2 per cent of all patients with venereal lesions show positive Wassermanns within fourteen days of the onset of the sore, and before the development of a typical luetic inguinal adenitis. After discarding all female cases and all in which the sore has been present over fourteen days, we found twenty-two with negative dark-field examinations and positive Wassermanns. The obvious interpretation to be placed on these findings is that of previous infection, which is borne out by the fact that we obtained a history of previous infection from eleven of these twenty-two cases.

The purpose of this note is to emphasize that people with syphilis may still contract venereal sores and that with negative dark-field examinations and delay in making a Wassermann test until the usual time for beginning positivity, a certain percentage will finally be labeled as chancres which are in reality chancroids superimposed upon a previous latent syphilitic infection. We, therefore, wish to call attention to the value of early Wassermanns and to urge the adoption of such examinations by those engaged in this work.

LABORATORY METHODS

AN HEMATOCRIT METHOD*

By C. M. Van Allen, M.D., New York

THE hematocrit is an instrument by which is determined the volumetric relationship between corpuscles and plasma in a specimen of blood. Separation of the two elements is brought about in the centrifuge by virtue of a difference in their specific gravities.

Since the invention of the hematocrit, in 1891, by Hedin,[1] a great many types and modifications have appeared. They may be classified for the purpose of description into two groups, depending upon whether minute amounts of blood are employed in a capillary tube, or larger amounts (5 to 15 c.c.) in a centrifuge tube. Some of the methods use whole blood and aim to complete sedimentation before coagulation takes place, and others inhibit coagulation to begin with by mixing the specimen with an anticoagulant.

The original hematocrit of Hedin is a capillary tube and has remained, with minor modifications, the most widely used of that type. It consists simply of a finely graduated glass tube about 10 cm. in length. The blood is collected in a separate container and mixed with a known proportion of isotonic anticoagulant solution (sodium sulphate 1 part; potassium bichromate 2 parts; water 100 parts), and a sample of this mixture is drawn up into the capillary tube. The ends of the tube are closed securely by fastening it in a specially constructed centrifuge head. Centrifugalization is then carried on at the rate of 10,000 rev. per min. for fifteen minutes or so, sedimenting the cellular content into a solid column at the outer end of the hematocrit tube. The height of this column in relation to the height of the total column occupied by the specimen represents the volumetric relationship of the corpuscles in the mixture examined. This ratio is then corrected for the amount of diluent added, to obtain the value for whole blood.

The other type of hematocrit is the more popular at the present time, chiefly because it can be homemade and is used with the ordinary power centrifuge. It consists of a graduated centrifuge tube. Ten or fifteen cubic centimeters of blood are drawn by venepuncture into a syringe and deposited in the tube with a quantity of anticoagulant, dry or in solution. After centrifugalization at high speed, the corpuscular sediment is measured upon the scale and corrected, if necessary, for dilution.

There is little purpose in entering upon description of the various modifications of these two types of hematocrit that have appeared, although many are very ingenious. None of them has gained any considerable popularity,

*From the Laboratories of The Rockefeller Institute for Medical Research, New York.
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and in each case the reason for this is to be found in a deficiency from one or both of two standpoints—practicability of operation and accuracy. Whole blood is not at all easy to handle and measure with precision. Its viscidity renders it somewhat adherent to the walls of a containing vessel, and difficulties readily arise in transferring a specimen accurately and cleanly from the collecting instrument (syringe or pipette) to the hematocrit tube. If the syringe is used for the purpose, the "dead space" in the extremity also complicates the technic. The slightest coagulation taking place in the specimen prevents complete sedimentation of the corpuscles, and in normal blood coagulation is initiated far sooner after shedding than the usual methods for estimation of clotting time would indicate. For instance, the wire loop method of Inchley[2] gives the clotting time of cat's blood as between 100 and 200 seconds, and by a wire loop method of my own[3] it is possible to detect coagulation in normal rabbit's blood as early as between twenty and thirty seconds after shedding. Apparently the Boggs and other commonly used procedures measure, not the initiation of clot formation, but the attainment of a certain degree of firmness or resistance in the clot, and subsequently their readings are taken well along in the process of coagulation. On account of interference by coagulation, the employment of whole and unaltered blood in the hematocrit necessitates having the centrifuge directly at hand, often an impracticable specification with the high-power machine. There is a distinct disadvantage, also, in those hematocrits which require larger specimens of blood than can be obtained readily by needle prick of the finger or ear lobe.

The physical principles underlying the operation of the hematocrit are well understood; nevertheless, many hematocrit methods have not in all respects held strictly to these principles. The most frequent inconsistency has to do with the use of an anticoagulant with the specimen of blood. The majority of investigators who employ an anticoagulant solution select the strength of the solution on a purely arbitrary basis. A very slight alteration of the osmotic pressure of the fluid medium surrounding red blood corpuscles results in a perceptible change in their volume, and care must be taken that the concentration of a diluent used in hematocrit determinations be such as to render it isotonic with the blood plasma. A common error arises also in the supposition that this difficulty may be overcome by the use of the anticoagulant in powdered form. Hooper, Smith, Belt, and Whipple[4] state that the addition to whole blood of the minimum amount (0.1 per cent) of sodium oxalate crystal that will completely inhibit coagulation changes the corpuscle volume by 3 per cent. Our own observations indicate that the addition of such an amount of the crystal to whole blood changes the corpuscle volume by 5.5 per cent. Most of those using a dry anticoagulant employ more than the minimum amount.

Sufficient detail in regard to the manner of centrifugalization is seldom given, and this is an important omission, because up to a certain point the degree of sedimentation is roughly proportional to the total centrifugal force exerted upon the corpuscles, and this force is a function involving several factors. In order to be sure of obtaining complete separation of the cor-

puscles from the fluid element, it is not sufficient to give as specifications for centrifugalization only the velocity and duration. The packing power that is obtained from one centrifuge can be duplicated in another only when, in addition to velocity and duration, are known the radius of the centrifuge head, the diameter of the hematocrit lumen and the dilution of the specimen. As will be demonstrated below, all of these factors play a perceptible rôle. Koeppe[5] has shown that, coincident with the attainment of an ultimate reading in the hematocrit, the red corpuscle mass assumes a peculiar translucency to transmitted light and at the same time an opacity to reflected light, and he suggests that this appearance is the result of the elimination of all interstitial fluid.

Very probably it is largely because of the complications of technic and the factors of error mentioned above, that the hematocrit has been very largely discarded from clinical use in favor of the hemacytometer, for certainly the information to be gained from the former instrument is of no less value than that rendered by the latter. Indeed, it would appear from what we know of the alterations in the size of the average erythrocyte that take place in the anemias, that volume determinations must represent erythrocyte function in pathologic conditions more truly than do cell counts. It is well recognized, for instance, that the physical vigor of a patient suffering from pernicious anemia and with one-quarter the normal number of red cells is considerably greater than that of a patient with a secondary anemia of like cell count. This is readily accounted for by the changes in erythrocyte size characteristic of these two diseases, for the blood of the first patient, when tested from the volumetric standpoint may easily be found to possess three-eighths of the normal amount of erythrocytic substance, and the blood of the second patient to contain only one-sixth. This advantage of the volumetric over the numerical estimation has been made of use in the prognosis of tuberculosis[6] and of other constitutional diseases.

However, there should be, of course, no competition between the hemacytometer and the hematocrit, for they measure quite different characteristics of the blood, each in itself of importance in understanding the status of the hemic system. This is brought out strikingly in the "volume index" of Capps,[7] which is the ratio between volume and numbers of red corpuscles in a specimen of blood. Haden[8] has recently emphasized the value of the volume index in the diagnosis of pernicious anemia. It is apparently more to be trusted than the color index. He asserts that a high volume index in association with achlorhydria is virtually pathognomonic of the disease even at a very early stage. This in itself is sufficient justification for including the hematocrit in the equipment of the clinical laboratory.

It has been necessary in some of our work to determine the relative corpuscle volume of rabbit's blood from small specimens and with considerable accuracy. A hematocrit method has been contrived which has given complete satisfaction from these standpoints under very extensive use, and which at the same time is simple of operation. No claim is made for originality in any single detail of the instrument or the method, for these are to be found in various of the hematocrits hitherto described, but so far

as can be found there is no hematocrit which combines these features or a technic which meets the requirements of simplicity and accuracy as adequately as does this. Because of the apparent need of a hematocrit of these characteristics in experimental and clinical work, the following description is given of the instrument and the method of its use together with sufficient study of the technical factors involved to insure reliable performance.

DESCRIPTION OF INSTRUMENT AND METHOD OF USE

The hematocrit is of the capillary type and is operated with either whole or altered blood. Sedimentation is performed in a power centrifuge.

Instrument.—Drawings of the instrument to be described are given in Chart 1, representing it ready for the collection of the blood specimen and, again, ready for centrifugalization.

The hematocrit is a glass pipette with a chamber, somewhat similar in design to the hemacytometer pipette. It is 10 cm. in length over all, of which the shaft occupies 7 cm. The bore of the shaft is about 0.5 mm. in diameter and should be perfectly uniform throughout. The shaft ends bluntly at its lower extremity and is graduated from the tip up to within a short distance of the chamber in a scale of 100 parts. The chamber is pear-shaped, larger end up, and has a capacity of about 1 c.c. The short length of tubing at the upper extremity of the pipette is also blunt tipped and serves for the attachment of the suction tube. The pipette is equipped with a rubber mouth suction tube. A rubber band, 7 to 9 cm. in length and 1 cm. wide, is employed for the purpose of sealing the ends of the pipette after collection of the specimen.

Method.—The hematocrit pipette is first prepared for use by attaching the suction tube. A free flow of blood upon the skin surface of the ear lobe or finger tip is obtained by needle prick and the first drop or two are discarded. As a fresh quantity appears it is drawn up into the bore of the pipette shaft to the top of the scale. Blood that adheres to the outside of the pipette is now quickly wiped away and, turning to a stock 1.3 per cent sodium oxalate solution, a small quantity of this anticoagulant is drawn with the specimen into the chamber of the pipette, about half filling it. Loss of fluid from the pipette is guarded against by placing a finger over the lower end of the tube bore, while the suction tube is removed and the pipette is enclosed from top to bottom with a rubber band, as illustrated in the drawing. No shaking or mixing of the specimen is necessary other than that which occurs inadvertently.

The pipette thus adjusted is placed in the centrifuge cup, shaft end down and properly counterbalanced, and centrifugalization is carried on at 2,700 rev. per min. or at any convenient rate above this for about fifteen minutes. Removing the pipette from the centrifuge at the end of this time, the volume reading of the red cell content is made directly, in percentage, from the shaft scale by noting the upper limit of the mass of red sediment. No correction for dilution is needed.

Provided the collection of the blood specimen has been accomplished rapidly enough to prevent clotting—and this is not at all difficult with a

little practice—the upper limit of the column of red cells to be found at the end of centrifugalization is sharply defined, and directly over it rests a thin layer of white substance. This layer is that commonly referred to as the "buffy coat," and it may be included with the red column in making the corpuscle reading, or not, as desired.

In place of sodium oxalate as an anticoagulant, powdered hirudin or powdered heparin may be employed. We have had no experience with these agents but suggest that the following procedure be used: A few particles of the powder are placed upon the skin at the spot chosen for puncture. Mixture of the agent with the specimen takes place automatically as the blood wells up from the wound. A measured portion of this altered blood is secured in the hematocrit as described above, but the blood is allowed to remain without dilution in the shaft bore. The pipette is enclosed in the rubber band and centrifugalized. Rotation at 2,700 rev. per min. for one hour should be sufficient to obtain a constant reading

Precisely the same technic as the foregoing may be employed without any form of anticoagulant. Considerable haste is necessary in the procedure, however, in order that the corpuscles may be separated from the plasma before clotting begins, and for this purpose the centrifuge must be directly at hand and all adjustments anticipated. This method probably gives the most reliable readings of corpuscle volume as there has been nothing introduced to disturb the osmotic pressure of the blood plasma, but it is not practicable for most purposes, particularly for clinical use.

Precautions in Technic.—Certain precautions should be emphasized in regard to the collection of the blood specimen. When drawing the blood into the pipette bore any considerable overflow past the mark should be avoided, for in reducing the level of the blood to the scale an appreciable quantity may remain adherent to the glass and give rise to too high a final reading. The best procedure is to allow the blood to enter to a point slightly above the mark and then, in wiping off the outside of the pipette with a gauze sponge, the tube bore may be touched lightly and the height of the blood column adjusted to the required mark. When the specimen has been accurately measured in this way, it is advisable immediately to draw the specimen on up the shaft a short distance so that when the tip of the shaft is immersed in the diluting solution none of the blood will be accidentally lost. This maneuver is feasible with this instrument because accuracy of dilution is not striven for. After the specimen is diluted with the anticoagulant solution there need be no further haste in the procedure.

The use of an ordinary rubber band to seal the ends of the pipette during centrifugalization has been found entirely satisfactory. The method is simple enough and there has never been evidence of leakage or other source of error attributable to it in routine use. There may be difficulty, however, from perforation of the band by the tube if the lower extremity of the pipette shaft is not fairly flat and smooth, or if centrifugalization is carried on at very high rates of speed.

RESULTS

Our use of the hematocrit has been confined to rabbits, and several hundred determinations have been made upon these animals, both normal and diseased. The data from 40 normal rabbits, picked at random from this material, are summarized in Table I.

Normal hematocrit values for the human being as given by Gram and Norgaard[9] are probably the most accurate available. The investigators used hirudin as an anticoagulant and a modified Hedin hematocrit. Sedimentation was accomplished at 3,000 rev. per min. for one and one-half hours. A summary of their data is given in Table I.

In the rabbit, under conditions of anemia, the relative red cell volume may be reduced to 18 per cent, and in man to 5 per cent.[8]

TABLE I

SPECIES		RABBIT	HUMAN				
Sex		Male	Male	Fe-male	Both Sexes	Both Sexes	Both Sexes
No. of Subjects		40	10	10	20	20	10
Relative Red Cell Volume	Maximum	54%	50%	43%	50%	51%	52%
	Minimum	33%	42%	39%	39%	37%	$41\frac{1}{2}$%
	Mean	39%	46%	41%	$43\frac{1}{2}$%	$44\frac{1}{2}$%	$48\frac{1}{2}$%
Method		Author's with 1.3% sod. oxalate	Gram and Norgaard with hirudin			Gram with 3% sod. citrate	Haden by method of Hooper et al. 1.6% sod. oxalate

EXPERIMENTAL STUDY OF UNDERLYING PRINCIPLES

Attention has been called, in the introduction of this paper, to certain important physical principles underlying the operation of the hematocrit. These principles have to do with the conservation of the natural size of the red corpuscles in the specimen of blood and with the attainment of complete sedimentation. Both objects are equally necessary in order that the readings obtained with the instrument shall truly represent the relative corpuscle volume as it exists in the circulating blood of the subject. The first object requires that the anticoagulant used shall not alter the osmotic pressure of the blood appreciably. If the anticoagulant is used in solution its concentration must be such as to be isotonic with the blood plasma, and if the agent is used in powdered form the amount added necessary to inhibit coagulation must be so small as not perceptibly to raise the osmotic pressure of the plasma. The second object requires that the centrifugal force employed to obtain sedimentation shall be sufficient to reduce the body of sediment to its ultimate size. The effect of centrifugal force in this instance is determined by several factors, among which are the speed, duration and radius of rotation, the distance through which the cells have to be driven, and the resistance offered to their passage.

The influence of each of these principles in the operation of the hematocrit has been studied experimentally, and upon the information thus afforded

have been based the specifications for the method of use of the instrument as given. The experiments are described herewith.

Strength of Anticoagulant.—Sodium oxalate solution was selected as the anticoagulant in our work. We have not been able to find accurate physico-chemical investigations determining the osmotic pressure of sodium oxalate solutions relative to that of the blood plasma. Hooper, Smith, Belt, and Whipple were likewise unable to obtain information on this point and solved the problem for themselves by using red corpuscle volume as an indicator of isotonicity between the two fluids in the following manner: Hematocrit determinations (syringe method described above) were made upon several sam-
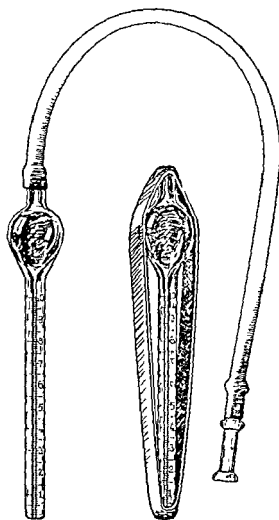
Chart 1.—The hematocrit pipette, equipped with mouth suction tube and sealed with rubber band ready for centrifugalization

ples of blood from the same animal (dog), using varying strengths of sodium oxalate solution as diluents. A determination of cell volume was also made upon a sample of the same blood, defibrinated and without dilution, as a control expressing the true relative corpuscle volume; and by comparing this value with the other readings it was possible to select the particular solution of sodium oxalate rendering a correct volume estimation. Two separate experiments of this sort agree exactly upon the required strength of sodium oxalate as 1.6 per cent. The data of one of these are reproduced in Table II, together with the results of another experiment which was performed as a check upon the work. The method of this third experiment was the same as of the others, except that, instead of using defibrinated blood as a control, the noncoagulable blood of a dog given a peptone injection was employed.

The data obtained were not full enough to determine an exact result but a very close agreement is indicated with the other experiments. These investigators, accordingly, adopted 1.6 per cent sodium oxalate solution in their work with the hematocrit.

While the agreement between the results obtained in the above experiments suggests the reliability of defibrinated and "peptone shock" bloods as controls of normal corpuscle volume, yet certain objections to these methods are justifiable. As regards defibrination, it would appear that in removing the fibrin from the blood a portion of the corpuscles is necessarily lost; and in "peptone shock" Descamps[10] has shown that the average size of the
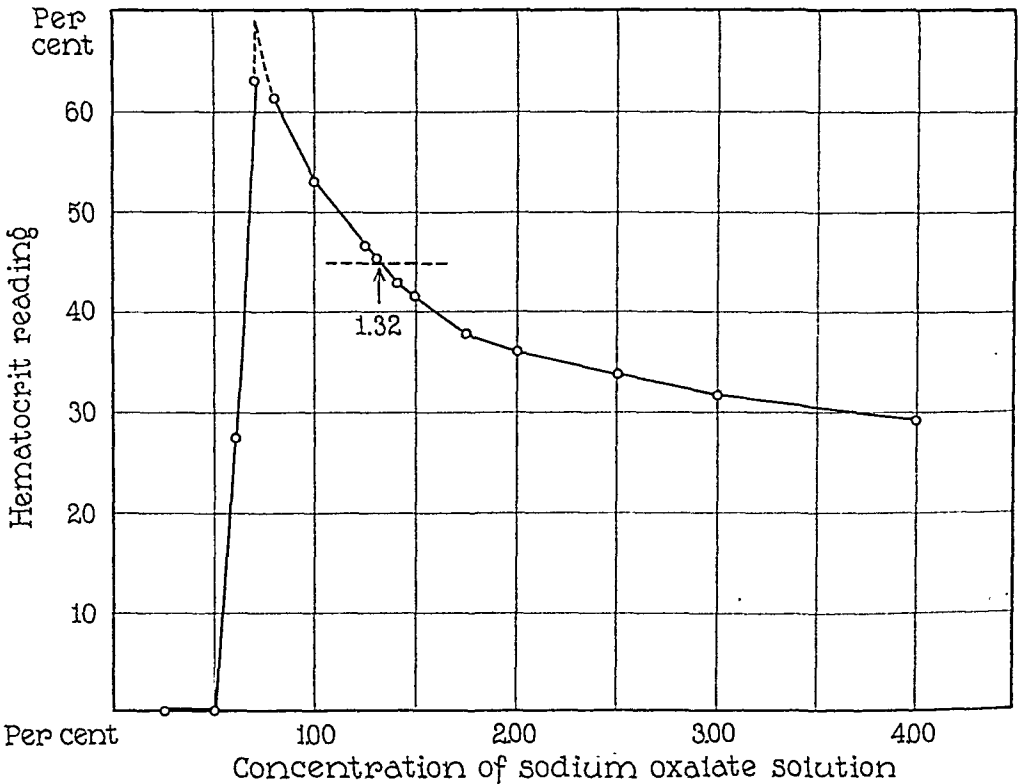
Chart 2.—Hematocrit reading of unaltered blood as compared to readings obtained upon the same blood treated with various concentrations of sodium oxalate in water.

erythrocytes is reduced. Accordingly, both types of blood used as controls for corpuscle volume would give values in terms of oxalate solution somewhat too high. However this may be, it is simpler to use for this purpose determinations made upon whole and unaltered blood.

We have repeated these experiments upon rabbit's blood, using the capilary hematocrit here described and unaltered blood as control. A series of readings were made upon the blood of a rabbit with sodium oxalate solutions of varying concentrations and, to compare with them, a "dry" reading upon the same blood (see technic given above).

The data of one of these experiments are represented graphically in Chart 2. The volume readings obtained with the various solutions are plotted

upon the chart and the points connected by a continuous line. The curve thus formed assumes a characteristic shape. Commencing with a volume of 0 per cent at 0.50 (per cent sodium oxalate), the curve rises sharply to 63 per cent at 0.70, and then begins to fall more and more nearly approaching the horizontal. The last reading, 29.2 per cent, was obtained from a 4 per cent solution. The reading of the hematocrit from unaltered blood ("dry" reading) is added to the chart as a horizontal dotted line at 45 per cent volume, and this line intersects the curve at a point corresponding to 1.32 per cent sodium oxalate.

Three other experiments of this sort have resulted by indicating 1.28, 1.32 and 1.44 per cent solutions of sodium oxalate as isotonic with the plasma of different normal rabbits. This variation in readings is probably not greater than the natural variation in osmotic pressures of the blood plasma of different normal individuals, and it is such that, should 1.30 per cent oxalate solution be employed routinely, the maximum deviation from the mean that would occur in the results on this account would be 1.25 per cent. In disease conditions with alteration of the osmotic pressure of the plasma beyond the normal range, there would arise greater error than this, but probably not so great as to exceed an allowable degree of inaccuracy. Should it be desirable at any time, because of osmotic pressure changes, to obtain more accurate results, the substitution of the "dry" in place of the "wet" method is to be relied upon. The degree of accuracy of this instrument, as indicated by these figures, is apparently considerably greater than that of the hemacytometer. In the latter instrument there enters the factor of personal equation to a considerable extent, which accounts largely for the variation often obtained between duplicate counts, even in skilled hands. Gulland and Goodall[11] place the maximum deviation from the mean reading with the instrument at about 11 per cent.

Force of Centrifugalization.—The centrifugal force exerted upon the cells in the hematocrit has been studied from the standpoint of its chief contributory factors, with the purpose of ascertaining the effect each exerts upon the process of sedimentation. From this information it should be possible to determine the conditions of centrifugalization which obtain complete sedimentation most economically and conveniently. Experiments are here described, consisting in hematocrit determinations upon rabbit's blood performed in the routine way with 1.3 per cent sodium oxalate solution, under conditions in which first one and then another of the factors affecting the centrifugal force is made to vary. The data are represented graphically in Chart 3.

Duration of Centrifugalization.—Three separate hematocrit determinations were made upon specimens of blood taken from the same rabbit by the routine method, centrifugalized at 2,700 rev. per min. Each specimen was read after three minutes of centrifugalization, after five minutes, after seven minutes, after eleven, fifteen, and twenty minutes, and these values were plotted to form curves representing the progress of the concentration of the corpuscle sediment.

These curves are reproduced in Chart 3 at B. It will be seen that they

TABLE II

STRENGTH OF SODIUM OXALATE	VOLUME READINGS EXPERIMENT 1	VOLUME READINGS EXPERIMENT 3
1.0	48.0	63.7
1.1	47.7	
1.2	47.4	
1.3	46.6	
1.4	46.1	61.1
1.5	46.3	
1.6	45.2	
1.7		56.6
	Defibrinated blood control	"Peptone shock" control
	45.1	59.6

behave in a fairly uniform fashion, descending together from left to right at a decreasing rate until at the fifteen minute point a level is reached that remains nearly constant. One may infer from this, that under the circumstances of the test as routinely performed centrifugalization for fifteen minutes is necessary in order to obtain a constant reading. The final values found in these three determinations were 33.0, 33.5, and 33.5 per cent respectively. This, by the way, is the usual degree of variation obtained in duplicate readings with this instrument.

Speed of Centrifugalization.—The preceding experiment was repeated upon another rabbit precisely in the same way, except that one specimen was centrifugalized at 2,000 rev. per min., another at 2,700 rev. per min., and the third at 3,600 rev. per min. The data secured were plotted as before.

The curves obtained are reproduced in Chart 3 at A. They conform to the type of those at B, but differ as regards the slope of descent. That is, at a speed of 2,000 rev. per min. (Curve 1) a constant value was reached after twenty minutes, at 2,700 rev. per min. (Curve 2) a constant value was obtained at fifteen minutes, and at 3,600 rev. per min. (Curve 3) the volume reading became constant from the eleventh minute on. There is a difference in the curves, also, as regards the final readings, for, while the levels of concentration reached by the sediment at the two higher rates of speed agree perfectly, at 2,000 rev. per min. the final reading of the corpuscles fell considerably short of the others.

It is to be inferred from this, that under the routine conditions of the test the rapidity of sedimentation increases with the rate of centrifugalization, and that the ultimate concentration of the sediment is also in proportion to the rate of centrifugalization up to a certain point (2,700 rev. per min.), above which rate there is no further change in the final reading.

Hematocrit Bore and Specimen Dilution.—In these experiments the hematocrit method differed from the routine procedure only as regards the diameter of the hematocrit bore and the degree of dilution of the specimen. An hematocrit was used, the shaft bore of which measured 4 mm. in diameter. Two determinations were made upon a rabbit; in one the specimen of blood was diluted by the anticoagulant to a degree approximately equal to that ordinarily employed in the capillary hematocrit, and in the other the dilution was at least five times as great (a degree impossible with the capillary instru-

ment). Readings of the two specimens were taken at various stages during centrifugalization as in the other experiments.

The curves constructed from the readings in these experiments are given at C in Chart 3. Curve No. 1 is from the highly diluted specimen; it reaches a level at fifteen minutes. Curve No. 2 is from the normally diluted specimen, and it reaches a level at five minutes. It is to be pointed out that the conditions underlying the determination of Curve No. 2 are identical in all respects with those of the curves at B except for the diameter of the bore of the hematocrit used.

It would appear, accordingly, that with other factors constant the rapidity of sedimentation is delayed by excessive dilution of the specimen of blood and accelerated by an increase in the diameter of the hematocrit bore, but that these effects are not to be expected as a result of the minor variations that might take place in the technic of the capillary hematocrit
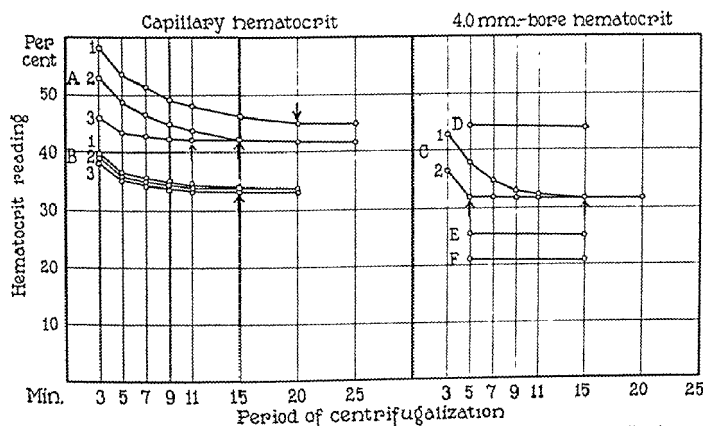
Chart 3.—Hematocrit readings obtained under various circumstances of centrifugalization.

Degree of Anemia.—The 4 mm.-bore hematocrit has been used for relative corpuscle volume determinations on rabbits in various stages of secondary anemia, and in some of these determinations readings were taken at five minutes as well as after the customary period of fifteen minutes of centrifugalizaton, for the purpose of testing the process of sedimentation in blood specimens of widely different corpuscle content. The same technic was employed as that used in the experiment producing Curve 2 at C. Three of these determinations are represented graphically in Chart 3 at D, E, and F. By comparing them with Curve 2 at C, it is readily to be seen that in various grades of anemia the process of sedimentation of the corpuscles in the hematocrit is not different from that which occurs in normal blood.

Radius of Rotation.—The question of the effect of variation in the radius of the centrifuge head upon the process of sedimentation of blood corpuscles

has not been approached experimentally. It is, of course, to be expected that variation in that factor would bring about changes in the centrifugal force. Since it has already been found that, provided the centrifugal force be kept above a certain amount and be allowed to act sufficiently long, an ultimate and constant volume of the corpsucles is reached, this factor may be disposed of, along with the questions of dilution and of tube bore, by laying down the following specifications for centrifugalization with the author's capillary hematocrit:

Centrifugalization of the specimen should be carried on at a rate of at least 2,700 rev. per min. for about fifteen minutes; and this without regard for the radius of the centrifuge head, providing it is greater than 9 cm., or for the slight variations that may occur in the diameter of the hematocrit bore and in the degree of dilution of the specimen.

DISCUSSION

In standardizing a hematocrit method for use in our work, as has been described, effort was made to procure a considerable degree of accuracy without greatly complicating the technic. The almost exact agreement that occurs between duplicate readings with the instrument has created confidence in its use in our hands as to uniformity of behavior, and this apparently is to be accounted for by the fact that the specimen is collected and measured under the same scale as is used for the final reading, eliminating entirely the necessity of a separate collecting instrument. Test of the physical principles underlying the operation of the hematocrit has assured a reasonable degree of conformity between its readings of cell volume and the actual volumetric status of the corpuscles in the circulating blood. The method should be suited to the various circumstances of the experimental or clinical laboratory. The pipette itself is the only special apparatus necessary; a convenient period of time is allowable between the collection of the blood and centrifugalization; and the requirements for sedimentation can be met by the standard power centrifuge.

There is one feature of the method that may need readjustment to new circumstances. The strength of the anticoagulant that we have used, 1.3 per cent sodium oxalate solution, is in agreement with the conditions of osmotic pressure of rabbit's blood, but it may not be the ideal concentration for use with the blood of another species. Before the test is employed upon the human being, for instance, it would be desirable to reinvestigate the question of the isotonicity of the anticoagulant solution, and this could be done simply by repeating the experiment depicted in Chart 2, it being necessary to employ only those strengths of the solution ranging within the zone of probable isotonicity with the blood. Readjustment of the anticoagulant for the species of animal would, indeed, be necessary in exacting work with the hematocrit, such as in determining total blood volume, but in the routine use of the instrument for the estimation of anemia, comparative values only are required, and here the use of the 1.3 per cent solution should be satisfactory. There would probably be some slight error in the readings obtained, but, whatever the error, its degree would be constant throughout. Those using the hematocrit

of Hooper, Smith, Belt, and Whipple for testing human blood have employed the strength of sodium oxalate which the authors had found suited to dog's blood, apparently without investigating its adaptability.

It would not be permissible to employ with this instrument a solution that has been shown to render correct readings with another hematocrit method. This follows from the fact that the adjustment of the concentration of an anticoagulant to a particular method means adjustment to secure accurate values of corpuscle volume in spite of the inconsistencies that the method may possess, such as the "dead space" of the collecting syringe, the disparity between the scale of graduations of the collecting instrument and that of the hematocrit, and incomplete sedimentation of the corpuscles. We have found, for example, that the syringe hematocrit technic (Hooper *et al.*) requires a 1.5 per cent solution of sodium oxalate when used for rabbit's blood, and that with this strength of solution it renders values identical with those obtained with 1.3 per cent sodium oxalate and the capillary hematocrit. Probably neither of these concentrations of the salt is actually isotonic with the blood of the rabbit.

In describing the experiments performed for the standardization of the present hematocrit method, and in drawing conclusions from them, it was stated that, after fifteen minutes of centrifugalization at 2,700 rev. per min. with a centrifuge head of 9 cm. radius, a constant volume of the sediment is attained. This is not strictly so. Greatly prolonged centrifugalization gradually reduces the corpuscle volume still further, and in order to obtain an absolute volume the process has to be carried on for an hour or more. This fact is responsible for the long periods of centrifugalization advocated by various workers, and it is for this reason, too, that we advise prolonged centrifugalization when the hematocrit is used without an anticoagulant or with an anticoagulant in powdered form. The inconvenience of such a procedure can be done away with, however, by adjustment of the diluting solution to such a strength that the volume of sedimented corpuscles obtained after a conveniently short period of centrifugalization, although not absolute for that particular specimen, is identical with the actual corpuscle volume as present in the circulating blood, by virtue of the fact that the surrounding fluid is somewhat hypertonic. We have selected a fifteen minute period, because the relative constancy of the sediment volume from this point on allows for a margin of inexactitude in the duration of centrifugalization, that so easily creeps into the routine performance of automatic work of this sort.

The employment of an anticoagulant in powdered form in hematocrit determinations is preferable in many ways to the use of solutions, provided the anticoagulant is so powerful as to be required in infinitesimal amount. Hirudin appears to answer this specification, for 0.001 gram of the substance is sufficient to inhibit coagulation in 10 c.c. of blood. The phosphatid, heparin, extracted from the liver by Howell[12] also exerts a very powerful anticoagulant effect, and its use in hematocrit tests has already been suggested by Mason.[13] Very obviously these substances, if used in minimal amounts in the blood specimen, would not affect appreciably the osmotic pressure of the plasma by increasing the salt content, but it is not certain that they may not

have such an effect by virtue of their other powerful characteristics. No one has determined this point experimentally. At present the principle obstacle to the employment of these agents with the hematocrit is their scarcity on the market, although it is now possible to obtain heparin in standard preparation.

The hematocrit may be used for the purpose of determining corpuscle fragility. As is graphically represented in Chart 2, with a decrease in the concentration of the surrounding fluid, the red corpuscles increase in size until the limit of distensibility is reached, when rupture takes place (laking), and corpuscle volume drops almost immediately to zero. The starting point of rupture is indicated quite precisely in the curve at 0.72 per cent of sodium oxalate solution. The determination of erythrocyte fragility by this method should be simple to carry out in routine work and of greater convenience than the double value commonly used as an index. With three or four stock solutions of strengths lying within the probable zone of corpuscle rupture and with as many hematocrit pipettes, a single centrifugalization would suffice for the estimation of corpuscle fragility upon an individual. Such a test was used some time ago by Hamburger.[14]

SUMMARY

It is believed that the hematocrit has not attained the degree of popularity as a clinical test that is justified by the significance of the information it reveals, and that the comparative obscurity into which the method has fallen is due largely to inadequacy from the standpoints of facility and accuracy.

Description is given of a new form of hematocrit and of the method of its use. Also, the principles underlying the operation of the hematocrit are critically discussed and put to experimental test, for the purpose of laying down specifications for the use of the instrument which produce reliable results and at the same time remain within the bounds of practicality.

REFERENCES

[1]Hedin, S. G.: Skandin. Arch. f. Physiol., 1891, ii, 135.
[2]Inchley, O.: Jour. Pharmacol. and Exper. Therap., 1921, xviii, 237.
[3]Van Allen, C. M.: Proc. Soc. Exper. Biol. and Med., 1924, xxi, 466.
[4]Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H.: Am. Jour. Physiol., 1920, li, 205.
[5]Koeppe H.: Arch. f. d. ges. Physiol. Pfluger, 1905, cvii, 187.
[6]Schlomovitz, B. H., Hedding, B. E., and Kehoe, E. J.: Jour. Am. Med. Assn., 1924, lxxxii, 1845.
[7]Capps, J. A.: Jour. Med. Res., 1903, v, 367.
[8]Haden, R. L.: Jour. Am. Med. Assn., 1924, lxxxiii, 671.
[9]Gram, H. C., and Norgaard, A.: Arch. Int. Med., 1923, xxxi, 164.
[10]Descamps, A.: Compt. rend. Soc. de biol., 1924, xci, 894.
[11]Gulland, G. L., and Goodall, A.: The Blood; a Guide to its Examination and of the Diagnosis and Treatment of its Diseases. Edinburgh and London, 1912, W. Green and Sons, pp. 360.
[12]Howell, W. H., and Holt, E.: Am. Jour. Physiol., 1918, xlvii, 328.
[13]Mason, E. C.: Surg. Gynec. and Obst., 1924, xxxix, 421.
[14]Hamburger, H. J.: Osmotischer Druck und Ionenlehre in ihrer Bedeutung für die Physiologie und die Pathologie des Blutes. Berlin, 1912, pp. 78. (Allgemeine Med. Verlagsanstalt, G. m. b. H.)

A CONVENIENT APPARATUS FOR THE DETERMINATION OF LOW CONCENTRATIONS OF CHLORINE IN CHLORINE-AIR MIXTURES*

By John H. Yoe, Ph.D., Charlottesville, Virginia

R ECENTLY an interesting paper† has appeared on the successful use of chlorine as a cure for colds, whooping cough, influenza, and other respiratory diseases in which the infection is located on the surface of the mucous membranes of the respiratory passages. In the use of this agent it is necessary to know, within fairly narrow limits, the chlorine content of the air-gas mixtures breathed by the patients. A simple apparatus, easy of manipulation and capable of accurately measuring the chlorine in dilutions of the order of a few parts (by volume) per million parts of air is of prime importance. Such an apparatus is described in the present paper with the thought that it may be of some assistance to physicians who may wish to try the chlorine treatment for certain respiratory diseases and who may not have access to more elaborate and expensive apparatus. Even when the simple portable apparatus described by Vedder and Sawyer† is used, it would seem advisable to check by chemical analysis the chlorine concentration from time to time; not only on account of possible variations in the amount of gas released by this apparatus, but also on account of the fact that the amount of chlorine lost by adsorption, diffusion, etc., will vary in different rooms. The chemical analysis, the present writer employed, is one of the standard methods for the determination of chlorine and there is nothing novel claimed for the apparatus. The apparatus is, however, simple in construction, easy to manipulate, and is capable of accurate measurement of chlorine in very low concentrations in air. It has been used in some experimental work (not with chlorine as a therapeutic agent) where it was necessary to determine accurately the chlorine content of air containing only a few parts (by volume) of chlorine per million parts of air. Since it was not practical to make the room even approximately air-tight and also since chlorine is rapidly adsorbed by the walls and various objects in the room, it was necessary to make frequent determination of the chlorine concentration. For these experiments the following apparatus and procedure were used successfully.

APPARATUS AND PROCEDURE

An aspirator bottle (2-liters capacity), (Fig. 1) is filled to the mark C with water. Forty or fifty c.c. of 4 per cent potassium iodide solution is put in vessel A and the screw pinchcock E opened and adjusted to a rate of flow of about one liter per minute. The chlorine-air mixture is thus drawn into the potassium iodide solution where the chlorine liberates an equivalent amount of iodine

which is soluble in the large excess of iodide solution. As soon as the water has reached the two-liter mark D, pinchcock E is closed and the absorption vessel A is transferred to the receiving bottle. The positions of the two bottles are then interchanged so that the receiving bottle now becomes the aspirator bottle, and vice versa, and the pinchcock E again opened. This procedure is followed until the desired volume of air-gas mixture has been drawn through vessel A, or if preferred, a large (ten-liter) aspirator may be used and thus avoid the interchanging of vessels. When a sufficient sample (say ten liters) of air-gas mixture
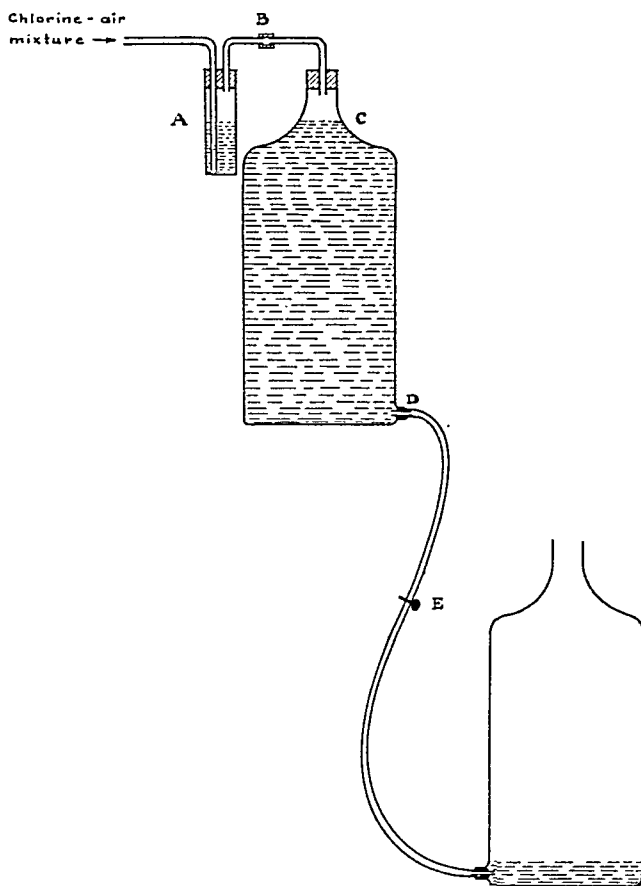
Fig. 1.

has been taken, vessel A is disconnected at B and the free iodine titrated with standard sodium thiosulphate solution, using starch suspension as an indicator. The results are calculated to parts (by volume) per million at 20° C. and 760 mm. pressure.

<center>SOLUTIONS</center>

1. Sodium thiosulphate solution (0.00166 N). .Dissolve about 0.3 gm. of sodium thiosulphate, $Na_2S_2O_3.5H_2O$, in a liter of freshly boiled distilled water and standardize against 0.0050 N iodine solution. Dilute the thiosulphate solution until the required strength of 0.00166 N is obtained.

2. Potassium iodide solution (4 per cent). Dissolve 40 gm. of potassium iodide in a liter of water.

3. Starch suspension. Add about 3 gm. of starch to one-half liter of hot water and boil for one hour. Filter while hot and keep in a bottle stoppered with a cotton plug.

CALCULATIONS

The standard thiosulphate solution is made of such a strength that each c.c. is equivalent to 20 parts per million (p. p. m.) of chlorine at 20° C. and 760 mm. in a one-liter sample. Then by taking a ten-liter sample, each c.c. of thio-
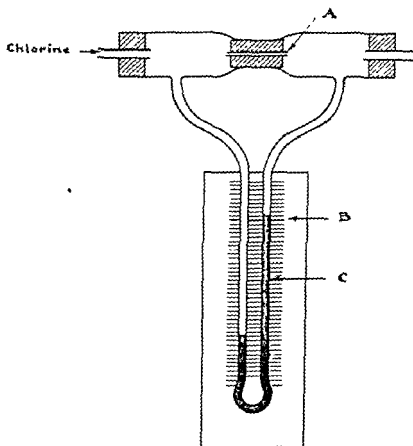
Fig. 2.—*A*, Small glass capillary tube through a rubber stopper; *B*, Millimeter scale; *C*, Water or oil manometer.

sulphate solution will be equivalent to two p. p. m. of chlorine. The method of calculation is as follows:

1 equivalent of chlorine $= \dfrac{24.04}{2} = 12.02$ liters at 20° C. and 760 mm.

1 liter of normal sodium thiosulphate $= 12.02$ liters of chlorine at 20° C. and 760 mm.

1 c.c. of 0.01 N sodium thiosulphate $= 0.1202$ c.c. of chlorine at 20° C. and 760 mm.

In a liter sample, 1 c.c. 0.01 N sodium thiosulphate $= 120.2$ p.p.m.

1 c.c. 0.00832 N sodium thiosulphate $= 100$ p.p.m. in a liter sample at 20° C. and 760 mm.

1 c.c. 0.00166 N sodium thiosulphate $= 20$ p.p.m. in a liter sample at 20° C. and 760 mm.

For a sample of X liters,

1 c.c. 0.00166 N sodium thiosulphate $= \dfrac{20}{X}$ p.p.m.

10 p.p.m. chlorine $= 0.03$ mg. chlorine per liter.

Obtaining the Chlorine Concentration.—The desired concentration of chlorine may conveniently be obtained from a cylinder of liquid chlorine to the valve of which is attached, by means of a short rubber tube, a glass capillary flow

meter (Fig. 2). The flow meter may be calibrated (see below) so that under a given head (preferably not less than 10 cm. of water) it delivers 50 c.c. of chlorine per minute. This would mean that a three-minute flow would be sufficient to give a chlorine concentration of approximately 5 p. p. m. (0.015 mg. per liter) in a room of about 1000 cubic feet capacity. To insure a rapid and even distribution of the gas, an electric fan should be placed near the outlet of the flow meter. Having introduced into the room the amount of chlorine to give approximately the desired concentration, i. e., 5 p. p. m., the exact chlorine con-
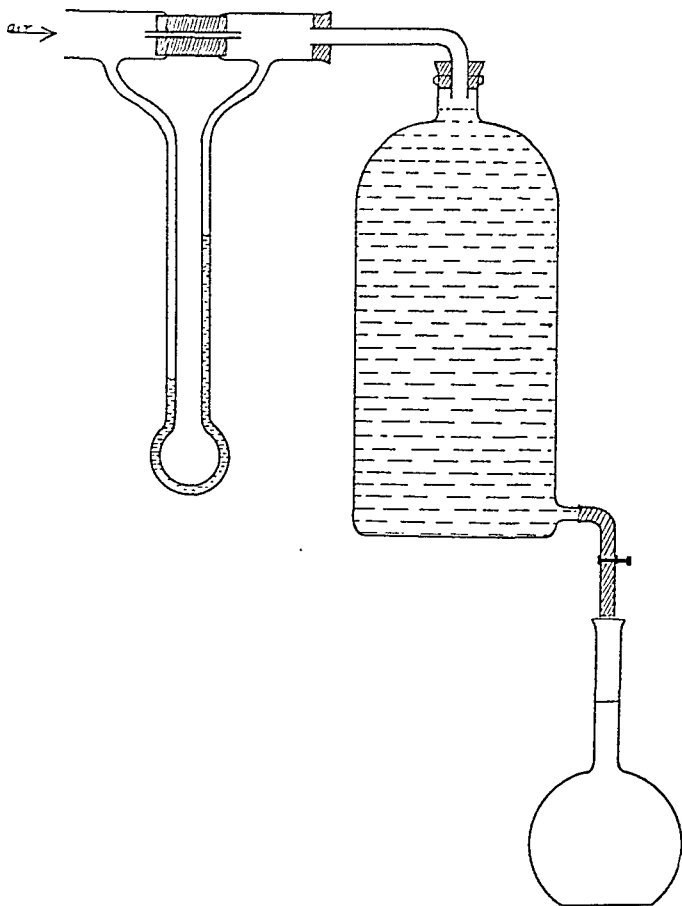
Fig 3.

centration is then determined by drawing a ten-liter sample of the air-gas mixture in the manner described above, under Apparatus and Procedure, and titrating the liberated iodine with standard sodium thiosulphate solution. As noted in the accompanying table, a titer of 2.5 c.c. of 0.00166 N sodium thiosulphate solution is equivalent to a chlorine concentration of five parts (by volume) per million at 20° C. and 760 mm. of pressure.

Calibration of Flow Meter.—The capillary tube A (Fig. 2), should be about 50 mm. long and should have a radius approximately 0.2 mm. The flow meter may be calibrated directly against pure chlorine but more conveniently against air, making the proper allowance for the difference in the viscosity of air and

TABLE OF EQUIVALENT DATA FOR A 10-LITER SAMPLE

C C. 0.00166 N Na₂S₂O₃ USED	CHLORINE P.P.M. (VOLUME) AT 20° C. AND 760 MM.	MILLIGRAM CHLORINE PER LITER	REMARKS
0.5	1	0.003	
1.0	2	0.006	
1.5	3	0.009	Odor distinct
2.0	4	0.012	
2.5	5	0.015	Desired concentration
3.0	6	0.018	
3.5	7	0.021	Slight irritation of throat
4.0	8	0.024	
4.5	9	0.027	
5.0	10	0.030	

that of chlorine. To calibrate against air, simply connect the flow meter to an aspirator bottle (as shown in Fig. 3) and find the difference in water levels in the two arms of the manometer that corresponds to an air flow of 40 c.c. per minute. The volume of air being drawn through the meter in any given length of time is obtained by noting with a stop watch the time required to fill a volumetric flask of, say, 500 c.c. capacity. Suppose the time is found to be 12.5 minutes and the difference in the height of the water in the two arms of the manometer is 10 cm.,—this would mean that with a 10 cm. head of water the flow meter will deliver 40 c.c. of air per minute or approximately 50 c.c. of chlorine per minute, since the ratio of the viscosity (at 20° C.) of air to that of chlorine is 1:1.25. Several readings with the stop watch should be made and the average taken. In each case, carefully adjust the flow of water from the aspirator bottle before putting the volumetric flask in place. This calibration is sufficiently accurate, since the concentration is finally determined by actual analysis of the air-gas mixture in the room.

AIDS TO URINALYSIS IN A LARGE HOSPITAL*

By C. Pons, M.D., and E. B. Krumbhaar, M.D., Ph.D., Philadelphia, Pa.

TO promote the better examination of urine in the central laboratory of a large hospital, we have found the following devices to be practical and useful.

I. *Urine Tubes and Transportation Rack:* Special urine tubes are provided, made of heavy glass, with spout and blunt conical bottom. They are 7½ inches long by 1¼ inches wide (outside) and have an approximate capacity of 90 to 100 ml. This promotes sedimentation, and permits convenient floating of a urinometer, with considerable saving of carriage space over the old sedimentation glasses. The transportation rack (Fig. 1) has 16 numbered apertures, corresponding to the numbered tubes, thus reducing the liability of confusion. The bottom of the tray contains felt or rubber matting to minimize breakage. Loaded
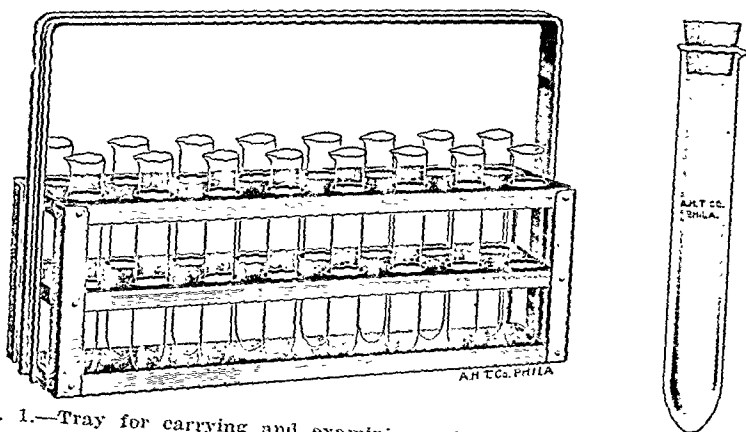
Fig. 1.—Tray for carrying and examining urine, with special urine tube.

racks are supplied to orderlies as the racks filled with specimens are brought in, each tube containing a few cubic centimeters of a 4 per cent solution of formaldehyde (i.e., same as used for tissue fixation, to prevent alkaline decomposition of urine) and securely corked. The laboratory slip is folded, wrapped about the tube and held with a metal clip. The advantages of this system are found to be that, (1) a uniform method of collection is provided; (2) curtailment of handling of the specimen in the laboratory; (3) decomposition is minimized; (4) centrifugation is as a rule unnecessary; and (4) the caliber and capacity of the test tube permits the specific gravity to be taken therein.

II. *Albumin Test:* For the heat and acetic acid test for albumin, Kolmer's wire rack is used, and sixteen ordinary medium sized test tubes of thin glass,

placed in close apposition in a row of eight each. These are two-thirds filled with urine and heated simultaneously with a fishtail burner, which covers five test tubes at a time. This not only causes a considerable saving of time, but minimizes boiling over and breakage. The rest of the test is performed in the usual manner. To promote the consistent reading by different internes, of the amounts of albumin present, Dr. Karr of our biochemical laboratory has prepared a permanent series of tubes corresponding to "faint trace," "trace," etc, up to "heavy cloud." These consist of sterile, formalinized gelatin, containing increasing amounts of albumin in the upper half of the tube and hermetically sealed. Against a black background these give a very fair comparison with the urine being tested. Lately we have adopted Purdy's[1] method for the detection of albumin, except that instead of using a 50 per cent solution of acetic acid,
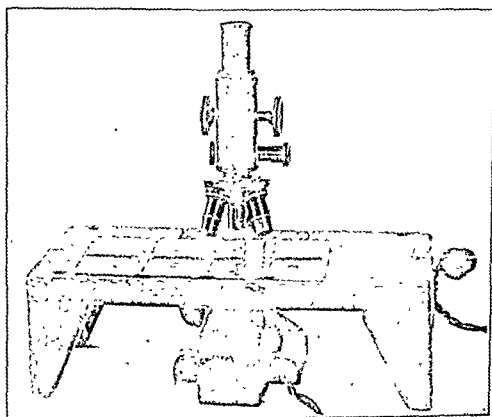
Fig. 2.—Special microscope stand and mammoth slide for examination of urine sediments.

we use a 25 per cent solution. We have found this test as sensitive as the heat and acetic acid test and practically no more trouble. Mucin, which is present in many urines, and which gives a positive test by other methods, is held in solution by the saturated sodium chloride, as used in Purdy's method. While we agree it is better to filter all urines for any albumin test, with this method necessary filtration is cut to a minimum.

III. *Qualitative Sugar:* Benedict's solution is used exclusively. Forty-eight tests are done simultaneously by immersing a Kolmer rack in a boiling water-bath for two and one-half minutes. The timing is important, as longer boiling gives some fallacious reductions

IV. *Quantitative Sugar:* For the last six months we have used Sumner's[2] dinitrosalicylic acid colorimetric method with the following advantages over the Benedict method in our estimation. Under our conditions, internes' errors are less frequent. It is not as expensive and there are fewer steps and

no calculations. It has the further advantage that any number of tests can be done simultaneously. Practically all the solutions used are placed in aspirator bottles to save time.

V. *Microscopic Examination:* To save time, without loss of accuracy, several pieces of ordinary windowpane glass, about 4 by 8 inches are prepared. These are divided by heavily painted lines into eight compartments each of which is about 2 inches square. The usual microscope stage is extended by a wooden table (as in Fig. 2), thus permitting the free movement of the mammoth slide under the objective. The urine from the conical ends of the original containers is pipetted off, eight at a time, a washed pipette being used for each specimen. As the pipette is simply a piece of tubing drawn to a blunt point a large number can always be available. Unless the high power is needed in an exceptional case, cover glasses are not needed, and the eight sediments can be examined long be-

Fig. 3.—Permanent albumin standards for urinalysis.

fore any harmful drying has taken place. The two inch square provides an extra large area for the accommodation of a large sample of urine.

Mr. Thomas Smull has aided us in the carrying out of these ideas.

A rotating rubber stamp, with a capacity of twelve letters, is used for the interne's signature. With over 100 urines daily this constitutes a considerable saving of time.

SUMMARY

1. Improved forms of urine collection tubes and transportation racks are described.

2. A quick improved method of performing the heat test for albumin is given, with permanent standards of comparison.

3. Improved qualitative and quantitative sugar methods are discussed.

4. A device for the quicker microscopic examination of urinary sediments, without loss of accuracy, is also described.

REFERENCES

[1]Purdy's Heat Test: Quoted in Clinical Diagnosis by Laboratory Methods. Todd, Fifth
    edition, p. 146, W. B. Saunders & Co., Philadelphia.
[2]Sumner, James B.: Jour. Biol. Chem., 1924-25, xlii, 287.

DEVICE FOR SAVING SUPERNATANT FLUIDS[*]

By Fred A. Wiggers, Toledo, Ohio

IT is essential in many laboratory procedures to save supernatant fluids. At times this entails the laborious task of pipetting off the fluid by hand. Especially is this true when the volumes are large. There has been no available apparatus in the market for accomplishing this procedure without losing the fluid into the drain pipes. Most of the mechanical decanting has been with the suction pump. With this method it is evident that the supernatant fluids are lost.

With the idea of saving much hand pipetting I devised, some years ago, a simple apparatus to collect supernatant fluids. This was accomplished by converting an ordinary straight-sided separatory funnel into a receiving funnel. By elaborating this scheme somewhat, a funnel was obtained that seems to meet all requirements. See Fig. 1.

In detail it is a separatory funnel with a closed roof, an inlet pipette for the intake of fluids, and a tube leading to the suction pump. In principle, the pump pulls a vacuum in the funnel, and this vacuum, in turn, pulls the fluid into the funnel. The entire operation can be aseptically carried out and the fluid preserved in a sterile condition. The funnel may be used for any class of work.

DISTILLING APPARATUS

Every laboratory has had the task of distilling and redistilling volatile fluids. Any such fluids are dangerous and great caution is required in distilling them. In most laboratories the usual procedure has been to distill them over from a flask resting in a water-bath. Such methods are cumbersome and dangerous.

Several years ago, I had a copper jacket arranged about a Florence flask and some water placed between the two. This method was not entirely satisfactory where an all-glass still was needed, and so the double-flask still (Fig. 2) was planned. This is simply a large flask completely incasing a smaller one, leaving a space for water between the two. In practice, water is allowed to enter through the pipette into the space between the flasks. This water is heated and the temperature regulated by a thermometer thrust into a suitable opening provided for it. Toward the top of the neck of the larger flask is a pet cock with a glass stopper to act as a safety valve for steam.
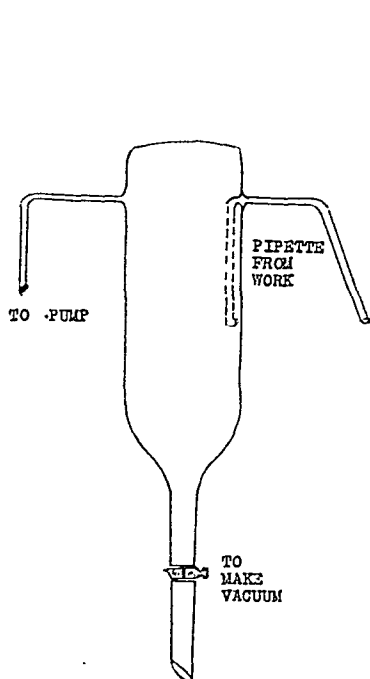
Fig. 1.
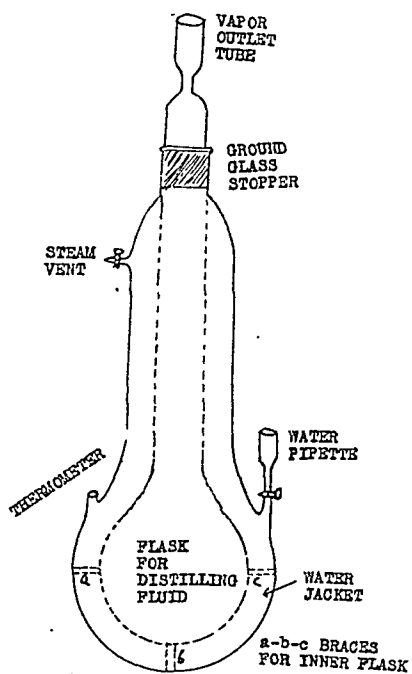
Fig. 2.
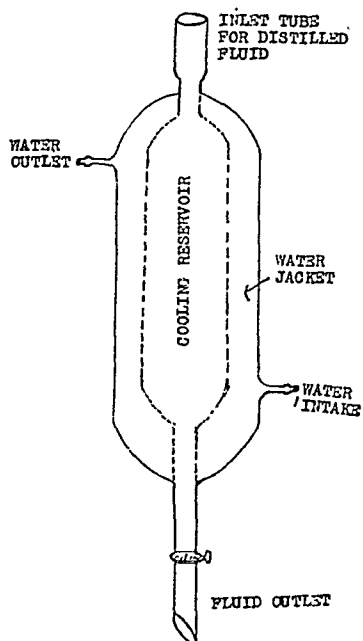
Fig. 3.

The neck of the inner flask rises above that of the larger one and is provided with an outlet tube for the vapor to be passed to the condenser. This outlet tube fits like a ground-glass stopper and the whole apparatus is easily cared for and kept clean.

COOLING RESERVOIR

In the redistillation of highly volatile fluids such as hydrogen peroxide, ether, gasoline, etc., there is always great danger of explosions. The apparatus pictured in Fig. 3 offers a solution for controlling the vaporization of the more highly volatile substances.

In principle, the apparatus is designed so as to confine the distilled fluid in a reservoir which is incased with an outer jacket through which running water flows. It is similar in this respect to that of a condenser. This running water has the effect of reducing the temperature enough so that volatilization is materially diminished. In practice the condensed vapor is received from the condenser and allowed to enter the reservoir. The connection between the condenser and the reservoir is made air-tight. Water is circulated through the outer jacket. When distillation is completed the apparatus is removed and the fluid allowed to flow out through the drawn-out stem below. A glass stopcock is, of course, provided to enable this removal. The entire apparatus is of glass.

No dimensions or capacities are given for either apparatus for the obvious reason that different sizes and capacities are needed for different laboratories.

The Journal of Laboratory and Clinical Medicine

EDITORIALS

Psychology and Anesthesia

NO group of drugs is more deserving of the grateful appreciation of all mankind than are those which are used to produce anesthesia or analgesia. A peculiar psychologic interest attaches to these substances in more ways than one. Many years ago Professor Jones had himself anesthetized with ether to the stage of unconsciousness seven different times in order that he might study at first hand, and in a strictly scientific manner, the strikingly interesting phenomena which ether is capable of producing in those who inhale its vapors. The experiments were carried out in a psychologic laboratory and special equipment and devices were provided so that observations could be made and recorded constantly throughout the course of the experiments. Trained attendants assisted Dr. Jones and made the necessary objective observations while recording the subjective sensations as they were described by Dr. Jones.

Here a phase of anesthesia which receives but little, if any, attention from the average medical man was studied and analyzed. The gradual fading away

of one's awareness of all earthly things and conditions was strikingly illustrated. The experience is somewhat similar to that of one who watches the receding shoreline as he stands on the deck of a vessel which is rapidly speeding out in the mists and fog of a rolling sea. One by one the special senses give way, each from moment to moment adding something to, or subtracting something from, one's mental contact with the outside world, with his own physical state or with his own inner consciousness. It is probable that nearly all patients, who are fully anesthetized, go through some such experience as this. But the conditioning circumstances under which the anesthetic is given, the mental character and attitude of the patient, and his previous notions of what should happen, have a great deal to do with his psychologic reaction to the effects of the anesthetic.

Dr. Jones, however, brought out an interesting feature of his experience,—a phase which apparently must have been reached a little while before total unconsciousness supervened. In this phase, which might be prolonged by proper administration of the anesthetic, all sensation was lost. Every special sense was cut off entirely from its connection with the inner consciousness. The mind, however, could still function and found itself freed from the usual restraints of the realities of life. As Professor Jones put it, he was in "the position of a disembodied spirit." Time and space no longer offered any obstacles, while physical infirmities and earthly longings had suddenly ceased to exist. Like a butterfly his soul could flit from star to star. What a splendid reality of unrealities;—what a satisfying physical demonstration of a state, which previously could have had only an imaginary existence, this experience might have been for some of the old philosophers in their attempts to found philosophic systems based on some such conception of the human mind as this. And perhaps the founders of some of our modern semireligious, semimedical systems might have profited by a study of some of the mental phenomena which anesthetics are capable of calling forth.

Let us carry the experiment a little farther. It is entirely possible in the laboratory to administer an anesthetic to an animal which may be presumed to pass through an experience resembling that produced in man. But if the anesthetic is pushed, by and by we reach a point where the respiration has stopped and the heart has just ceased to beat. So far as the animal is concerned it is as dead as it will ever be. Exactly this same thing may, and indeed frequently has happened in the case of man. However, under these circumstances, it is usually possible by the use of appropriate means promptly to revive the animal. An injection of adrenalin solution directly into the cavity of the left ventricle, artificial respiration carried out by means of a suitable mechanical device, and careful massage of the heart, may usually be counted on to readily restore the animal. Obviously, with similar treatment, the same thing would happen in man. Here we have Professor Jones' experiment carried to the last degree. In those few moments when the man has ceased to breathe and his heart no longer beats, has he indeed become a disembodied spirit or is he still a living soul? When only three drops of chloroform, or a cubic centimeter of adrenalin solution, stand between man's soul and a fathomless future on the one hand, or a hard cold world on the other, just what may his status be presumed to be? In

these days when so many people are trying to find out whether evolution is or is not evolution, we should like to propound to these same people, the question as to what is the status of the soul of a man who is no longer living but is not yet dead.

It is interesting to note that there are styles and fashions in anesthesia the same as there are styles in clothes. Forty and fifty years ago chloroform was very popular. Most of the young surgeons who got their training in those days clung to chloroform faithfully until they ceased to operate or else were literally forced to give up chloroform by a younger generation of surgeons. Then ether came in style and finally nitrous oxide with oxygen. But the styles changed slowly and were in reality very good indices of the rate at which one generation of surgeons can shove the preceding one out of the field of operations.

But now we have another of these psychologic, style-changing contests before us. Ethylene has but recently been introduced by Luckhardt as a new anesthetic. It has a large field of usefulness before it. For many types of operations, and in a great variety of conditions, it undoubtedly possesses advantages over any other general anesthetic now in extensive use. Does this mean that ethylene will be widely and rapidly adopted in the surgical clinics of the land? By no means. If we may draw any conclusions from the development of anesthesia in the past we may well conclude that only by another generation will ethylene be utilized to the fullest extent of its possibilities. And while everyone recognizes the danger of its explosibility still it now seems probable that this danger will soon be reduced to a minimum. It is not this feature that will delay the introduction of ethylene, but it is in reality the psychologic limitations which God has prescribed for surgeons (anesthetists?), and perhaps for a number of others who do, or should, use anesthesia in their work. But there is only one possibility that can remove ethylene from the sphere of its future usefulness, and that is the possibility that some still more satisfactory anesthetic may be found to take its place.

—D. E. J.

The Diagnostic Significance of Red Blood Cell Volume

THE estimation of the size of the red blood cell is now recognized as a most valuable diagnostic and prognostic measure in the study of anemia. On the basis of differences in size, classifications of the anemias, which represent fundamental cell properties, are possible. "Size" may be expressed in terms either of diameters or of true volume. It is preferable to determine the actual cell volume, since the thickness of cells is as variable as the diameter or even more so. One not uncommonly finds cases of pernicious anemia in which there is a marked increase in average cell volume without significant change in diameter as a result of the marked increase in thickness.

The first determinations of cell volume were made by Hedin, in 1893,[1] using the hematocrit of Blix. Herz,[2] in a series of cases, counted the red cells simultaneously with the determination of the volume of cells in a known amount of blood. He described a factor represented as the "mean volume of a single cell"

which he found quite variable in disease. To Capps,[3] however, is due the credit for making the first accurate study of average cell volume and applying clinically the facts so learned. Capps introduced the term "volume index" which represents the volume of the average red blood cell in terms of normal cells and affords a simple means of comparison.

In estimating the volume of red cells, Capps employed the small hematocrit tubes of Blix, which give only the relative mass. A simpler and perhaps more accurate method[4] is to centrifuge 10 c.c of blood mixed with 2 c.c. of isotonic sodium oxalate (1.6 per cent). In this procedure the absolute volume of the cells is obtained. Van Allen[5] has recently suggested a modified micro tube which necessitates no special apparatus other than the tube, but also gives only relative readings. In calculating the volume index only a red cell count is necessary in addition to the relative or absolute cell mass. Capps pointed out that in pernicious anemia the volume index is greater than 1.00, while in secondary anemia it is 1.00 or less. This has been amply confirmed by other observers.

Price-Jones[6] and other English observers have determined the "size" of the red cells in terms of diameter. Five hundred cells on a stained film are drawn in outline, magnified a thousand times, and measured with a millimeter scale up to 0.5 mm. The mean diameter of the five hundred cells is taken to represent the average diameter of the blood cells of the person tested. Pijper[7] has suggested an optical method for determining the cell diameters utilizing the blood film as a diffraction grating. Such procedures are, however, much more laborious and time-consuming than the method used in the determination of the volume index. They fail to show also the true size in these cases in which there is a marked increase in thickness with consequently much greater cell volume but relatively little change in diameter.

The size of the average cell is remarkably constant. The average volume index is always approximately 1.00 in normal adults. The normal average mean diameter is 7.24 micra.[6] In absolute terms the actual average cell volume is 9.6 by 10^{-11} c.c.[8]

The English observers agree that the average mean diameter is always much increased in pernicious anemia. Passey[9] records similar results in sprue, Dibothriocephalus anemia, and in anemia produced experimentally in rabbits by the injection of the toxin of hemolytic streptococci. Any deviation from the normal in other secondary anemias is manifested as a decrease in size. Such differences suggest the separation of anemias into megalocytic and nonmegalocytic groups, which represent fundamental cell differences. There is much to suggest that the volume increase in the group so affected represents the action of a toxin common to the various clinical conditions in which it occurs.

Price-Jones points out that the red cell diameters are independent of the degree of anemia in pernicious anemia. The increase in size may be apparent at the onset when the degree of anemia is slight. This fact has been emphasized also in studies of volume index. In fact, in pernicious anemia the volume index may be much greater in the early stages when the count is relatively high than in the later stages when the count is low, thus indicating its value as an early diagnostic test. The conditions other than pernicious anemia in which the

average cell size is increased are infrequently observed and can be readily differentiated clinically. A definite increase is presumptive evidence of pernicious anemia.

Knowledge of the cell volume is also of the greatest value in the prognosis of secondary anemia. Increase in cell volume must precede the increase in the hemoglobin content. Given two patients with low hemoglobin, improvement is necessarily more rapid in the one having the average cell volume nearest normal.

REFERENCES

[1]Hedin, S. D.: Skandinav. Arch. f. Phys., 1890-91, ii, 360.
[2]Herz, Max: Virchow's Arch., 1893, cxxxiii, 339.
[3]Capps, J. A.: Jour. Med. Res., 1903, p. 367.
[4]Haden, R. L.: Arch. Int. Med., 1923, xxxi, 766.
[5]Van Allen, C. M.: Jour. Am. Med. Assn., 1925, lxxxiv, 202.
[6]Price-Jones, C.: Guy's Hosp. Rep., 1924, lxxiv, 10.
[7]Pijper: Lancet, 1924, ccvii, 367.
[8]Haden, R. L.: Folia haematol, 1925, xxxi, 113.
[9]Passey, I. D., and Braine, J. F. C.: Guy's Hosp. Rep., 1924, lxxiv, 329.

—R. L. H.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

Practical Anaesthetics*

THIS book is a classic in brevity, conciseness, and accurate information. The details and pertinent facts of general, local, infiltration, regional, intrasacral, spinal, and splanchnic anesthesia and analgesia are all "practically" covered. A well-written history is condensed into six pages.

"Ethanesal, a preparation of mixed ketones dissolved in purified ether to the strength of 5 per cent" is urged to take the place of ether, and it should if the authors are correct. This opinion is based upon the practical effects of ethanesal as observed by Boyle and his associates in over 10,000 cases: "Less taste and smell, consequently less irritation, and quieter respiration, more analgesic, fewer after effects, as vomiting."

In reviewing the local anesthetics in use, it is stated that, "On the whole, novocaine appears to be the best combination of efficiency and low toxicity."

The book seems especially adapted for the busy surgeon in addition to being a valuable handbook for the anesthetist.

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